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Annual Report of Program Activities

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National Institute
of
Allergy and Infectious
Diseases

Annual Report of Program Activities

October 1, 1981 - September 30, 1982

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

For Administrative Use

TABLE OF CONTENTS

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1982 ANNUAL REPORT

Director 1-1
Immunology, Allergic, and Immunologic Diseases Program 2-1
Microbiology and Infectious Diseases Program 6-1
Extramural Activities Program 11-1

Intramural Research Program

- Office of the Scientific Director 16-1
- Laboratory of Biology of Viruses 17-1
- Laboratory of Clinical Investigation 18-1
- Laboratory of Immunogenetics 19-1
- Laboratory of Immunology 20-1
- Laboratory of Immunoregulation 21-1
- Laboratory of Infectious Diseases 22-1
- Laboratory of Microbial Immunity 23-1
- Laboratory of Molecular Microbiology 24-1
- Laboratory of Parasitic Diseases 25-1
- Laboratory of Viral Diseases 26-1
- Rocky Mountain Laboratories
  - Laboratory of Microbial Structure and Function 27-1
  - Laboratory of Persistent Viral Diseases 28-1
  - Epidemiology Branch 29-1
  - Rocky Mountain Operations Branch 30-1

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES*

1982 ANNUAL REPORT PROJECT NUMBER LISTING

Z01 AI

00006-11 LMM
00011-17 LVD
00013-19 LMM
00018-16 LMM
00019-07 LVD
00027-15 LMM
00030-14 LI
00035-07 LI
00036-17 LI
00037-15 LI
00040-08 LI
00043-17 LCI
00045-14 LCI
00047-13 LCI
00048-12 LCI
00057-09 LCI
00058-08 LCI
00061-20 EB
00063-12 EB
00065-09 LMSF
00069-21 LMSF
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00073-17 LPVD
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00076-24 LMSF
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00099-12 LPD

Z01 AI

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00108-11 LPD
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00131-15 LMI
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00138-08 LMI
00139-17 LMI
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00147-07 LI
00148-07 LI
00154-07 LCI
00155-07 LCI
00160-06 LPD
00161-05 LPD
00162-06 LPD
00166-05 LIG
00167-05 LIG
00168-05 LIG
00169-05 LIG
00170-05 LIG
00171-05 LIG
00172-05 LIG
00173-05 LIG
00180-04 LIG
00182-04 LMSF

*Does not include terminated or inactive projects.

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1982 ANNUAL REPORT PROJECT NUMBER LISTING

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00183-04 LMSF
00185-03 LPD
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00194-03 LMSF
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NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1982 ANNUAL REPORT PROJECT NUMBER LISTING

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NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1982 ANNUAL REPORT PROJECT NUMBER LISTING

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ANNUAL REPORT OF PROGRAM ACTIVITIES
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
October 1, 1981 through September 30, 1982

Fiscal Year 1982 can be characterized as one of increasing scientific opportunities for the NIAID community in the presence of tight fiscal constraints. Some of the scientific opportunities are being attributed to "the biologic revolution," based upon recombinant DNA technology, hybridoma technology and an increasing understanding of the immune system.

Dr. Richard Krause continues to direct the Institute in a dynamic fashion, presenting testimony on behalf of the Institute to the Congress, speaking to professional societies and voluntary health associations, and contributing his advice within the Department as well as at international forums.

The Director's speaking engagements this year included:

> "Commentary from the National Institutes of Health." October 31, 1981. Presented before the Association of Professors of Dermatology, Washington, D.C.
>
> "Outreach: The NIH Mandate and Perspectives." November 19, 1981. Presented at the Conference of the AADC and CIRID Program Directors, Chevy Chase, Md.
>
> Remarks introducing Dr. Stanley Cohen, Kinyoun Lecturer. December 3, 1981. Wilson Hall, NIH, Bethesda, Md.
>
> "Is the Biological Revolution A Match for the Trinity of Despair?" February 13, 1982. Presented at the 1982 Alumni Seminar on Science and Our Society. University of Georgia, Athens.
>
> Opening Remarks at the State-of-the-Art Symposium on Occupational Immunologic Lung Disease. February 25, 1982. Washington, D.C.
>
> "There is a Balm in Gilead." Commencement address. May 26, 1982. Presented at Case Western Reserve University School of Medicine, Cleveland, Ohio.
>
> "Virus Infections: The Persistent Challenge." July 3, 1982. Presented to the Rockefeller University Board of Trustees and the Rockefeller University Council, Rockefeller University, New York City.
>
> "The Persistent Challenge of the Microbial World." June 14, 1982. Presented at Merck Sharp & Dohme International Medical Advisory Council, Paris, France.

A book of essays, The Restless Tide -- The Persistent Challenge of the Microbial World, was published this year, based on lectures and speeches of Dr. Krause's since he became NIAID director in 1975. A feature article, containing the views expressed by Dr. Krause in the book and in an interview, appeared in the Science News section of the New York Times. Dr. Krause and Dr. Karl Western, Assistant Director for International Research, were co-authors of a chapter on Bacterial, Spirochaetal and Rickettsial Diseases, which will appear in a text, Tropical and Geographical Medicine edited by Drs. K. Warren and A. Mahmoud.

The Director continued to serve as chairman of the Biomedical Sciences Scientific and Technical Review Committee (called BIOS) at the World Health Organization in Geneva. He also participated as a member of the U.S. delegation to the U.S.-Japan Cooperative Medical Science Program meeting in Tokyo in July. The Institute signed an interagency agreement with the U.S. Agency for International Development for a 5-year project on the epidemiology and control of vector-borne diseases in the Near East. The project involves the cooperation of American, Egyptian and Israeli scientists in studies on Rift Valley Fever, malaria and leishmaniasis, three diseases of considerable public health importance. Dr. Western serves as project officer of this Near East effort. In addition to periodic visits to the Near East, he visited India this year to review NIAID bilateral studies in India, and has fostered an active Institute program in tropical medicine and international health.

The Institute was fortunate in recruiting Dr. Bernard Talbot to succeed Dr. John R. Seal as Deputy Director on October 1. As Special Assistant to the Director of the National Institutes of Health since 1978, Dr. Talbot had been deeply involved in the original development and subsequent revisions of the NIH Guidelines for Recombinant DNA Research. He continued this activity in addition to his newly acquired responsibilities as NIAID Deputy Director.

NIAID staff received significant honors: Dr. Krause received an honorary Doctor of Science degree from Hahnemann Medical College and an honorary Doctor of Laws degree from Thomas Jefferson University. He also was named Distinguished Alumnus of the Year by Case Western Reserve University School of Medicine. Dr. Robert Chanock received the Robert Koch Prize (Germany). Dr. William E. Paul was elected to the National Academy of Sciences. Dr. Michael Frank received the Distinguished Service Medal, highest award in the PHS Commissioned Corps. Drs. William S. Jordan, Jr., and Norman Salzman received Senior Executive Service awards for outstanding performance.

Office of Program Planning and Evaluation

The major documents for which the Office of Program Planning and Evaluation (OPPE) had lead responsibility were the Institute's Annual Research and Evaluation Plans and their associated reports. The office also responded to numberous requests from trans-NIH committees, HHS offices, congressional staffs and public and professional groups for program information. The office assisted in preparing background information for Congressional budget presentations.

During the year a reorganization of the OPPE was approved and the office now has four sections (Planning, Evaluation, Classification, and Data Control). In January, Dr. C. David Wise joined the office at the completion of his Grants Associate training year and will serve as Head of the Evaluation Section.

Office of Recombinant DNA Activities

The NIH Guidelines for Research Involving Recombinant DNA Molecules continued to evolve as major actions recommended by the Recombinant DNA Advisory Committee (RAC) were approved by the Director, NIAID. The RAC met on February 8-9 and June 28, 1982. Major revisions of the Guidelines was promulgated in the Federal Register on April 21, 1982, and on August 27, 1982. Under these revisions, the Guidelines continue to be mandatory for institutions receiving NIH funding. Section III of the Guidelines, which specifies containment conditions for covered experiments, has been reorganized and greatly simplified. Certain experiments continue to require prior review by NIH, certain experiments continue to require review by an Institutional Biosafety Committee (IBC), and certain experiments require notice to an IBC simultaneously with initiation of the experiment. Appendix B, which is a classification of microorganisms on the basis of hazard, was originally a reprint of the CDC classification issued in 1974. In the latest revision, the CDC classification has been revised by the RAC for the purposes of the Guidelines. The previous requirement for NIH approval of certain large-scale experiments, i.e., greater than 10 liters of culture, has been eliminated; such experiments can now proceed after IBC approval. The Director, NIH, had accepted a recommendation of the RAC to establish a Large-Scale Review Working Group. The Working Group met on February 9 and June 29, 1982. At these meetings, the Working Group reviewed a draft Report of the CDC/NIOSH *Ad Hoc* Working Group on Medical Surveillance for Industrial Applications of Recombinant DNA. The report examines three areas of concern: (1) exposure to recombinant DNA-containing microbes, (2) exposure to the products of recombinant organisms, and (3) exposure to reagents used in producing processes. The Working Group suggested some changes in the report which will be considered by the full RAC at its October 1982 meeting.

ORDA continued to interact with other Federal agencies regarding recombinant DNA research and associated activities. The Director, ORDA, serves on the Department of Agriculture's Recombinant DNA Committee, which met on a frequent basis during FY 82 to coordinate USDA policy decisions with NIH. The Director, ORDA, served as a lecturer and discussion leader at a seminar on Science, Technology, and Public Policy sponsored by the U.S. Office of Personnel Management at its Executive Seminar Center in Oak Ridge, Tennessee, on September 24, 1982.

Issues of the *Recombinant DNA Technical Bulletin* were published in December 1981 and in March, June, and September 1982. In July 1982, DHHS and the Office of Management and Budget renewed approval for publication of the *Bulletin* for an additional two years. Detailed minutes of RAC meetings were prepared and distributed using ORDA's extensive mailing keys.

ORDA continued to interact with the international scientific community during FY 82. ORDA hosted a Workshop on Introduction of DNA into Eukaryotic Cells sponsored by the U.S.-Japan Cooperative Program for Recombinant DNA Research in Bethesda on November 9-10, 1981. ORDA served as U.S. coordinator for a Workshop on Host-Vector Systems Other Than *E. Coli* sponsored by the U.S.-Japan Cooperative Program in Tokyo on June 3-4, 1982. The Director, ORDA, attended, as an invited guest, the annual meeting of the Committee on Genetic Experimentation of the International Council of Scientific Unions at the Cold Spring Harbor Laboratory on March 12, 1982. ORDA completed plans for a presentation on the NIH Guidelines at a U.S.-Japan Joint Workshop on DNA Safety to be sponsored by the Japanese Institute for Total Safety. ORDA also completed plans for a Workshop on Gene Transfer in Eukaryotic Cells to be sponsored by the U.S.-Japan Cooperative Program for Recombinant DNA Research. Both workshops will take place in early FY 83.

ORDA presented information on recombinant DNA research and the NIH Guidelines at a symposium on genetic engineering at the University of the Redlands in Redlands, California, on October 28, 1981, at a course on Industrial Applications of Genetic Engineering sponsored by the American Law Institute-American Bar Association in Washington, D.C., on October 30, 1981, and at the University of Maryland in Baltimore on May 20, 1982. The Director, ORDA, testified before the California Assembly Health Committee's hearing on genetic engineering in Sacramento, California, on December 1, 1981. Within the NIH community, ORDA provided orientation talks on the NIH Guidelines and NIH policies to NIGMS staff on April 26, 1982, to staff of the Division of Safety on July 7, 1982, and to the NIH Extramural Associates Program on February 9 and August 3, 1982.

Office of Research Reporting and Public Response

The ORRPR had a productive year fulfilling its mission to publicize and to provide information about NIAID programs and research efforts in the fields of allergy and infectious diseases. Major highlights included: the release of the 28-minute public education film, "Jennifer, a Revealing Story About Genital Herpes"; completion of the slide show, "Coping with your Allergies, At Home, At School and on the Job"; publication of the booklet, "Lupus Erythematosus: A Handbook for Physicians, Patients and Their Families"; a new exhibit designed for the White House Conference on Aging; increased press activity; and the record number of ORRPR publications distributed this year.

Response to the film about genital herpes has been overwhelming since the initial announcement in March. By the end of the fiscal year, it is estimated that "Jennifer..." will have been seen by nearly 150,000 persons in schools, medical centers, hospitals, doctors' offices and community groups all over the U.S. and at numerous military installations abroad. Media interest has also been extensive with excerpts of the film appearing on the NBC Nightly News, and appearances of the film's "stars" on the PBS McNeil-Lehrer Report, Morning Break, WDVM-TV Health File and other TV and radio shows.

Technical editorial assistance was provided by ORRPR staff on Dr. Krause's book, The Restless Tide: The Persistent Challenge of the Microbial World. Editorial assistance also was given on the text, Cholera: The American Scientific Encounter, co-authored by former NIAID Deputy Director, Dr. John R. Seal, and Dr. William van Heyningen of Oxford, England. Staff reviewed approximately 400 manuscripts prepared by NIAID scientists and administrators for submission to professional journals or presented at scientific meetings. Four articles were written for News and Features and seven for Search for Health columns, both NIH publications that are sent to a broad media audience. About 40 stories were submitted to the NIH Record.

Scientific meetings and exhibits -- A redesigned exhibit on allergies and immunology, which includes an asthma slide show, attracted hundreds of visitors at the American Academy of Pediatrics meeting. A new vaccine development slide/tape show, produced for the White House Conference on Aging, was devoted to areas of special concern to senior citizens. In addition, ORRPR staff assisted in publicizing three NIAID meetings -- "Allergy for Primary Care Physicians," a "Family Conference on Asthma and Allergies" and the "Occupational Immunologic Lung Disease" symposium.

Press activity -- Increased press activity generated by the ORRPR press liaison resulted in response to more than 450 inquiries from reporters and writers on a variety of subjects. "Notes to Editors" were sent to media representatives alerting them to the latest research accomplishments of Institute scientists and grantees including the use of acyclovir for the treatment of genital herpes, the identification of the organism suspected of causing Lyme disease, and the isolation and characterization of two antigens specific to *Mycobacterium leprae*, the causative organism of leprosy. The FDA licensing of acyclovir and the hepatitis B vaccine led to news stories which cited the research contributions made in these areas by NIAID supported scientists. Two interviews with Dr. Krause by New York Times' reporters resulted in a feature article entitled, "Despite Conquests, War on Infections is Far From Over" and an article in the New York Times magazine section about genital herpes. Another interview led to a feature article on immunology carried by the Des Moines Register's national syndicate. The Chief of NIAID's Allergic Diseases Section was quoted extensively in the lead issue of American Health, which took an in-depth look at allergies. Background data was supplied to the Associated Press for national articles on the common cold, and publicity was provided for the NIH Consensus Development Conference on Defined Diets and Childhood Hyperactivity. In July ORRPR press liaison, Joan Hartman, left to become Director of Public Information, Johns Hopkins University, Homewood Campus, Baltimore.

New Publications -- "Lupus Erythematosus: A Handbook for Physicians, Patients and their Families," was published by the NIAID and the Lupus Foundation of America in a cooperative effort, with extensive editorial and technical assistance given by ORRPR staff on text, design and layout. Revised editions of 6 ORRPR publications were issued this year -- fact sheets on genital herpes, common cold and rabies and pamphlets on drug allergy, insect sting allergy and poison ivy allergy.

Distribution of publications -- An all-time record of approximately 350,000 copies of publications were distributed by ORRPR this year. Accounting for a majority of the requests was an article carried in the October 1981 issue of Good Housekeeping Magazine listing the availability of several of the publications. Material was also furnished for scientific meetings, health fairs, and the annual Minority Biomedical Science Seminar.

Congressional reports -- A special report on sexually transmitted diseases was prepared for the Senate and House Appropriations subcommittees as well as special "add-on" reports on NIAID's research related to arthritis, cystic fibrosis, diabetes, digestive diseases and genetics.

Public and Congressional inquiries -- ORRPR staff responded to approximately 650 inquiry letters and more than 4,500 telephone calls including 60 Congressional inquires and 70 Freedom of Information requests.

Liaison with volunteer groups -- The Asthma and Allergy Foundation of America's move to Bethesda has afforded an opportunity for even closer ties than in the recent past, particularly in press relations and public information. A joint review of audiovisual productions on allergies and asthma was conducted with the goal of coordinating efforts. Close cooperation continues with the American Social Health Association in our common goal of prompt dissemination of current information about sexually transmitted diseases.

1982

Annual Report
Immunology, Allergic and Immunologic Diseases Program
Table of Contents

Report of the Director....2-1

Allergy and Clinical Immunology Branch

I. Administrative Summary....3-1
  A. Scope....3-1
  B. Organization....3-1
  C. Awards and Support Levels....3-4
II. Scientific Summary....3-5

Genetics and Transplantation Biology Branch

I. Administrative Summary....4-1
  A. Scope....4-1
  B. Organization....4-1
  C. Awards and Support Levels....4-2
II. Scientific Summary....4-2

Immunobiology and Immunochemistry Branch

I. Administrative Summary....5-1
  A. Scope....5-1
  B. Organization....5-1
  C. Awards and Support Levels....5-3
II. Scientific Summary....5-4

REPORT OF THE DIRECTOR
IMMUNOLOGY, ALLERGIC AND IMMUNOLOGIC DISEASES PROGRAM
Fiscal Year 1982

A. Administrative Summary

1. Organization and Functions

The Immunology, Allergic and Immunologic Diseases Program (IAIDP) was initiated on October 1, 1976, coincident with the reorganization of NIAID into major programs characterized by special professional functions. With this change the IAIDP assumed the responsibility for research, training, and conference activities that formerly were assigned to the Allergy and Immunology Branch of the Extramural Programs and contract activities in transplantation immunology and immunologic research resources previously administered within the Collaborative Research Program.

During FY 1982, the IAID Program operated according to the following organizational plan:

Office of the Director (OD)

Director-Sheldon G. Cohen, M.D.
Deputy Director-Bernard W. Janicki, Ph.D.
Special Assistant to the Director-Dorothy D. Sogn, M.D.
Program Officer-Judith G. Massicot, Ph.D.
Assistant for Program Management-Kathy Duvall

Clinical Applications Section (CAS)
Head-Daniel I. Mullally, M.D., M.P.H.

Allergy and Clinical Immunology Branch (ACIB)

Chief-Robert A. Goldstein, M.D., Ph.D.
Program Associate-Janet Ayres

Immunology and Immunochemistry Branch (IIB)

Chief-Bernard W. Janicki, Ph.D.
Program Officer-John G. Ray, Jr., Ph.D.

Genetics and Transplantation Biology Branch (GTBB)

Chief, Henry Krakauer, M.D., Ph.D.
Program Associate-Justina S. Grauman, M.S.
Serum Bank Manager-Anne Heath

Each of the three IAIDP Branches assumes responsibility for administering initiated research grants and collaborative research contract awards, developing programmatic initiatives and new projects, serving on trans-NIH coordinating committees and taking active roles in the development and conduct of workshops and conferences in areas of biomedical and biological disciplines and the clinical medical specialities with which they are concerned.

All three Branches are also involved with programs related to Research Career Development Awards and training, i.e., individual and institutional Fellowships and the Young Investigator Research Grant Awards designed to assist and encourage investigators in early stages of their careers to develop research interests and capabilities in immunology. In addition, the Allergy and Clinical Immunology Branch administers Allergic Disease Academic Awards which are designed to further the careers of mid-level investigators on career tracks in academic and research allergy, and this year initiated the Clinical Investigator Award. The purpose of this new program is to provide opportunities for young individuals trained as clinicians with demonstrable interest and aptitude in the fields of immunology and allergy, and in turn to increase the pool of clinical investigators applying knowledge and relevant basic biomedical sciences to clinical problems in allergic and immunologic diseases.

The IAID Program also assists investigators by procuring and providing certain reference standard and research reagents not otherwise available from commercial sources. These have included human and mouse histocompatibility typing sera, purified allergens, and antisera to lymphocyte subsets. Program staff's responsibility and awareness of needs to assist constituent fields are reflected in the expansion and development of several new endeavors in this area.

2. Centers and Program Projects

The IAIDP/NIAID Clinical Research Centers Programs are now comprised of 22 Centers. Eighteen Asthma and Allergic Disease Centers (AADC), located at the Scripps Clinic and Research Foundation, Mayo Clinic, NIAID NIH Clinical Center, and university medical school and hospital centers at California/San Francisco, Colorado, Northwestern, Kansas, Tulane, Johns Hopkins, Harvard/Brigham and Women's Hospital, Creighton, New York (NYU), SUNY Buffalo, SUNY Stony Brook, Duke, Texas/Dallas, Wisconsin and Tufts, were in operation during FY82. The Centers at Creighton, SUNY Buffalo, Scripps Clinic and Research Foundation and Kansas terminated during FY82. The Centers at SUNY Stony Brook and Wisconsin successfully competed for renewal and two additional centers were approved at Rockefeller-Cornell University Medical Center and Medical College of Wisconsin.

The 4 Centers for Interdisciplinary Research on Immunologic Diseases (CIRID) at the university medical school and hospital centers at California/Los Angeles (UCLA), Georgetown, Washington (St. Louis) and Rochester (N.Y.) have undergone an initial special three-year program review. In addition to the conduct of basic biomedical research and clinical investigations in asthma, allergic and immunologic diseases and the application of basic data to attacking clinical problems in diagnosis, prevention and treatment of patients with these disorders through multi-disciplinary collaborative approaches, these Centers have been focusing efforts on the development of educational and community activities, outreach regional programs for both professional groups and the laity.

Through the provisions of the Lymphocyte Biology Program the IIB has set the goal of attaining comprehensive knowledge of the life history of immunocompetent cells and of the genetic and phenotypic factors that determine cellular fate and function *in vivo* and *in vitro*. Program project studies (Lymphocyte Biology Centers) in this defined area are in progress at five universities located at Rockefeller, Yeshiva, Texas/Southwestern (Dallas), Harvard and Jewish

Hospital of St. Louis. The Yeshiva and Jewish Hospital centers successfully competed for renewal while the applications of Rockefeller and Harvard are currently in the review process for competing renewal.

To encourage investigations of the underlying mechanisms of immunologic disease and to enhance basic knowledge relevant to the etiology, prevention and management of such disorders effective from one of two disciplinary approaches, clinical immunology or immunopathology, the ACIB has established a special program, Mechanisms of Immunologic Diseases. Program projects were operational at university medical school and hospital centers at Harvard (Children's Hospital), Johns Hopkins, Scripps Clinic and Research Foundation, Northwestern and Stanford. At its January meeting, the Advisory Council approved an additional program project in this category at the University of Alabama/Birmingham.

In supporting projects in transplantation immunology, the objective of the GTBB is to take advantage of the momentous advances in technical procedures and in the understanding of the molecular cellular processes to bypass remaining hazards associated with immunosuppressive therapy and rejection of organ transplants. Focus is given to the application of the most up-to-date concepts and techniques of immunology to the evaluation of the immune system of recipients in all circumstances attendant to transplantation. Of especial concern is clarification of immunoregulatory balance and modulation of immunologic activity. The first Program project in this area was awarded to Johns Hopkins Medical School and Hospital.

By annual announcements (RFA) new institutions are invited to compete for available funds with ongoing Centers at the time of submission of renewal applications. An additional feature of this Centers - Program Project endeavor has been inter-Branch facilitation and support of investigator exchanges between institutions comprising this network. Rapid developments and progress in research on physiologic function, chemistry and the genetics of the immunocompetent cells have not only been rewarding in revealing fundamental knowledge but additonally have pointed to relevant leads for effecting clinical relevance. Accordingly, staff exchanges are designed to favor applications of basic biomedical science data to clinical investigations.

3. Special Research Grant Announcements

During FY 1982 IAIDP issued the following Requests For Research Grant Applications (RFA):

a. To allow for continuation of ongoing programs described above.

Asthma and Allergic Disease Centers
Projects in Mechanisms of Immunologic Disease
Program Projects in Lymphocyte Biology
Centers for Interdisciplinary Research on Immunological Diseases

b. To encourage development of new projects in special areas

Program Projects in Transplantation Immunology (described above).

Mechanisms in Food Allergy. The objective of the ACIB in these studies is to effect research directed at the elucidation of basic mechanisms essential for an understanding of the role of food antigens in causing a variety of allergic and/or adverse reactions. By addressing basic immunologic and/or other pathophysiologic mechanisms of causation it is anticipated that this important but underserved clinical area can be accomplished. Grants were awarded for proposals submitted in response to this RFA to the University of Chicago, Department of Psychiatry and to Duke University, Department of Pediatrics.

A new award mechanism was begun in FY82, the Clinical Investigator Award. Its purpose is to provide promising young physicians with demonstrated aptitude in research the opportunity to develop fully into independent investigators under the supervision of appropriate mentors and thus increase the pool of physician investigators who will apply their training to clinical problems in allergy and immunologic diseases.

c. ACIB sponsored joint Institute Announcements for cooperative research initiatives:

ACIB-sponsored joint Institute Announcements for research initiatives related to: the immunological aspects of diabetes, Immunology of Diabetes, with NIADDKD; immune mechanisms of cutaneous disorders, Immunodermatology, with NIADDKD; and immune responses in the lung underlying interstitial pulmonary fibrosis with NHLBI.

NIAID, with NIADDKD, will recruit physicians trained in clinical endocrinology for placement in NIAID supported immunology postdoctoral training programs in order to prepare them for research careers in the immunologic and immunopathologic aspects of diabetes.

NIAID is collaborating with NIADDKD in that Institute's Special Emphasis Research Career Award (SERCA) program in diabetes mellitus. This award provides the opportunity for a physician with developing research interests to acquire multidisciplinary experience in basic and clinical areas needed to study the metabolic, endocrinologic and immunologic aspects of diabetes mellitus.

4. Collaborative Projects

The Hybridoma Cell Line Bank contract awarded to the American Type Culture Collection has entered the distribution stage and is continuing with acquisitions. 222 shipments of cell lines have been sent to 57 investigators; 42 cell lines are available now for distribution; 39 cell lines are being processed and 10 lines are being held pending further study for usefulness.

The ACIB continued developing its resource capabilities by acquisition, storage and distribution of reference standard materials including: a battery of fungal antigens and human antisera relating to the identification of etiologic factors of hypersensitivity pneumonitis, selected lyophilized preparations of food antigens for standardized challenge testing in clinical studies on food allergy, a complement (C3) standard for biologic assays, and in collaboration with WHO an antihuman IgG-horseradish peroxidase agent for use in the enzyme linked immunosorbent assay (ELISA). This ACIB effort by quality control is supported by a contract awarded to the Mayo Foundation for operation of an Allergen Resource Reference Laboratory.

By contract support the GTBB has continued to assist in the work of the International Bone Marrow Transplantation Registry maintained at the Mt. Sinai Medical Center in Milwaukee. Data collection is designed to provide pertinent information on immune processes and immunologic problems associated with bone marrow transplantation and thus represents a unique resource to the medical science and clinical communities coming under the GTBB's purview.

The clinical trial of skin testing with major and minor penicillin derivatives in hospitalized adults is now being extended into its third year. Reagents are available for testing at each of 8 participating university medical school and hospital centers located at California/Los Angeles (UCLA), Washington (Seattle), Northwestern, Colorado, Rochester, (N.Y.) Cornell, Walter Reed Army Medical Center and the Clinical Center NIAID-NIH. At issue is whether skin testing with major and minor determinants of penicillin can predict hypersensitivity reactions to administration of penicillin (or its analogs) in history positive and in history negative hospitalized adults. If this predictive value is shown, the study may further demonstrate whether a positive or negative history adds information in this respect, beyond that gleaned from skin tests. Of the required 500 history positive patients, 467 have thus far been enrolled. 82% of such patients are skin test negative and may receive penicillin without danger of immediate life-threatening allergic reactions. Of the required 3500 history negative patients, 437 patients have thus far been enrolled. 4% are skin test positive leading to the assumption that such patients are at high risk for immediate life-threatening allergic reactions to penicillin.

Renewal support was given for continuation of the trans-NIH contract project on tabulation and analysis of immunoglobulin sequence data under the direction of Dr. Elvin Kabat, who joined the NIAID this year in the capacity of Expert (immunochemistry). The object of this project is to publish sequence data into a single reference resource, to develop further concepts concerning immunoglobulin synthesis and diversity, and to expand the data base to include nucleic acid sequences of immunoglobulin chains and the inclusion of sequence data on HLA, H-2, Ia, and related molecules.

A collaborative study at the Sidney Farber Cancer Institute is concerned with investigation on immunodepressant effects of a series of conventional drugs including corticosteroids, cytotoxic agents, antilymphocyte/thymocyte serum or globulin and total lymphoid irradiation. Indications have been obtained that some of these agents do selectively depress subsets of T lymphocytes. This can serve as the first step in the elucidation of the mechanisms of action of these conventional immunodepressant drugs and agents. In addition these data provide the insights that will be needed for the proper employment of subset specific monoclonal antibodies as immunomodulating agents. In conjunction with certain pilot studies on the use of these agents being conducted presently, within a very short time it may be possible to conduct a full scale clinical trial of these monoclonal antibodies in transplantation and in a variety of autoimmune disorders.

Requests for Proposals (RFP)

During 1982 the following RFPs were either issued or responses reviewed in order to initiate new projects and further ongoing work in identified areas of need and to invite competition for awards for ongoing work at the time of submission of renewal applicant proposals.

Preparation of alloantisera specific for H-2 murine cell membrane antigens: To provide a continuous source of well characterized, functionally active H-2 alloantisera for distribution to qualified investigators and for quality control testing of other acquired antisera; awarded to Mayo Foundation.

Development of methods to prepare bulk supplies of alloantisera specific for mouse cell surface determinants. To continue the development of methodology for the production of alloantisera specific mouse cell surface antigens, the preparation of relatively small quantitis of such antisera, the storage of these reagents and their distribution to selected qualified investigators, and the provision of quality control testing of other acquired antisera; awarded to Sloan-Kettering Institute for Cancer Research.

Screening, Characterization, and Characterization of Histocompatibility Testing Antisera Capable of a More Effective Definition of Transplantation Antigens of the Ethnic Subpopulations in the United States: To overcome difficulties in tissue typing of noncaucasians, an outcome that is unsatisfactory from the theoretical immunogenetics standpoint and in terms of its practical consequences in organ and tissue transplanation; awarded to Georgetown University and the Sloan Kettering Institute.

Development and clinical evaluation of a rapid cellular cross matching technique suitable for use in cadaveric organ transplantation. To develop a cellular cross matching technique sufficiently rapid to be useful in estimating histocompatability between recipient and cadaveric donors in renal and other organ transplantation and to supplement incomplete information determined by serologic cross matching for free formed antibodies; to be awarded.

Allergen resource reference laboratory. To acquire and make available standardized materials, allergens and other immunopharmacologic reagents, for clinical investigations and to serve as a reference laboratory for the production, analysis, assay and quality control of a variety of immunologic-allergenic reagents and materials and as a centralized reference laboratory facility for immunodiagnostic tests; awarded to Mayo Foundation.

Request for contracts to effect Master Agreements concerned with the development and supply of serologic reagents for the investigation of polymorphic human cell surface antigens was announced.

5. Research Resources

The IIB has provided qualified researchers with the highest possible specificity and purity antisera to available cellular antigenic determinants for basic immunologic investigations of mouse systems: Ia, Ly, H-2, Thy-1, Qa-1 and Tl. Support of this programmatic interest is a development of the recognition of the role of murine strains as the focal point of research in experimental immunogenetics because of the effect of this series of closely linked genes on tissue transplantation, susceptibility to viral infection and cell interactions during immune responses. 107 ml. were distributed to 62 qualified investigators, in 100 shipments. There are 11 antisera ready for distribution and 16 under development.

A newly developed immunologic H-2 complex reagent has been made available to grantees and qualified investigators studying manipulation of the immune mechanisms by antigen recognition control and immunotherapy through the work of the IIB. Newer specificities H-2 antisera related to tissue histocompatability and specific Ia antisera involved in immune response mechanisms have been identified. Ly antisera specific for thymus dependent lymphocytes and for nonhistocompatability cell marker studies, tumor specific antibodies and theta cell antisera have been developed, banked and distributed. 825 vials of alloantisera were distributed to 107 qualified investigators in 105 U.S. and 30 foreign shipments.

A manual comprising various tissue typing techniques, methods for freezing tissue cells and the acquisition of antisera is available and has been provided to qualified researchers and medical libraries.

An author-journal index of literature concerned with hybridoma technology, basic research and catalog of hybridoma strains has been developed and maintained by the IIB.

6. Histocompatibility Serum Bank Activities

The inventory and ordering procedures for bank sera and the evaluation of reference laboratory reports is functioning on an automated system. The work of the Serum bank has involved 364 sera offers, 140 of which represented rare specificities, and 560 users.

A data acquisition and analysis capability in the serum bank has been developed for the purpose of conducting inhouse studies on pooled national data in the area of immunogenetics.

The production of redesigned typing sera trays has been completed and their release effected. These comprise basic clinical trays and an extended tray to meet research needs. More recent approaches to DR serum typing indicative of promising advancements has resulted in the design and production of DR locus typing tray. Laboratories working in this newly developing area serve as sources of appropriate serum sample contributions. The serum bank distributed approximately 70,000 of the new trays to U.S. and foreign laboratories during FY 1982.

Reevaluation of needs and usage of homozygous typing cells has resulted in redefinition of contracts for their acquisition.

7. Outreach and Community Activities Programs

Within the research framework and resources of the NIAID supported Asthma, Allergic and Immunologic Diseases Centers (AADC'S and CIRID's), the IAID program has encouraged and fostered the development and support of outreach and community activities programs by centers' staffs. Among the highlights resulting from this programmatic interest were the following projects:

UCLA-CIRID - offered instructional self-management programs for asthmatic children

NIAID Clinical Center, Georgetown CIRID, Johns Hopkins AADC, Metro Washington D.C. chapter Asthma and Allergy Foundation of America (AAFA) and the National Jewish Hospital and Research Center/National Asthma Center at Denver--held a conference on Practical Applications of Allergy for Primary Care Physicians and a public meeting on Asthma and Allergies.

Rochester CIRID - conducted instructional programs in satellite community hospitals by visiting center staff in an effort to improve awareness, diagnosis and management of immunologic diseases within these clinical settings.

Northwestern AADC - established a national Asthma/Allergy hotline maintained within the Executive Office of the American Academy of Allergy and Immunology (AAAI) in Milwaukee.

A number of Centers have worked with local chapters of the Lupus Foundation of America (LFA) and the AAFA in developing their public information and educational programs for patients and interested laity. Especially to be mentioned are: the CIRID's at Washington (St. Louis), Georgetown and UCLA, and the AADC's at Harvard, Tufts, Duke, SUNY Buffalo, Northwestern, Johns Hopkins, Scripps, Kansas City, Mayo and N.Y.U.

8. Awards

Dr. Sheldon G. Cohen, IAIDP Director, received the AAFA Distinguished Service Award in recognition for his years of service as a member of the National Board of Directors of the Foundation on October 30, 1981.

Dr. Robert A. Goldstein, ACIB Chief, received the NIH Award of Merit in 1981 for his imaginative, innovative and effective leadership in developing community activities and expanding new areas of investigation within the NIAID Allergy and Immunology Centers.

Dr. Bernard W. Janicki, IIB Chief, was the recepient of a high quality performance award from the Director of the NIAID.

Dr. Henry Krakauer, GTBB Chief, received a PHS commendation medal.

Dr. Elvin A. Kabat, of Columbia University and currently serving in the position of Expert at NIAID, received the Philip Levine Award from the American Society of Clinical Pathologists for his contributions of quantitative immunochemistry to the knowledge of blood group antigens; the Docteur Honoris Causa by the Universite d'Orleans in France for his work on the structure of blood group A,B,H, Le and I substances; the Doctor of Philosphy Honoris Causa from the Weizmann Institute of Science, Rehovoth, Israel, in recognition of his contributions to immunochemistry, his achievements regarding the nature and size of antigenic determinants and his recent development of a unique computerized approach for the analysis of the genetic basis of antibody complementarity; and the Columbia University 1982 Award for Excellence (in pure science).

Dr. Harden W. McConnell, an NIAID grantee at Stanford University, received the Dickson Prize for Science from Carnegie-Mellon University for his contributions to the development and application of magnetic resonance techniques to chemical and biological problems and for his more recent contributions to the field of membrane biophysics and cellular immunology.

Dr. Peter Doherty, and NIAID grantee at the Wistar Institute, and Dr. Rolf M. Zinkernagel, an NIAID grantee at the University of Zurich, were selected to share the Paul Ehrlich and Ludwig Dannstaedter Prize for 1983 from the Paul Ehrlich Foundation for their contributions to understanding the genetic restrictions affecting immune responsiveness.

Dr. D. Bernard Amos, an NIAID grantee at Duke University, received the 3 M Life Sciences Award at the annual meeting of the Federation of Societies for Experimental Biology for his contributions in describing and clarifying the major histocompatibility complex's role in controlling immune function.

9. Special Staff Projects

The report of the Clinical Application Section's epidemiologic study based on medical records at Children's Hospital National Medical Center of D.C. is in the process of publication. A ten fold increase has been noted over the past 25 years in the diagnosis of asthma as a leading cause of admissions among children not under continuing outpatient care. Further study will be undertaken to evaluate whether there may be demonstrable relationships to identifiable socioeconomic, environmental or etiologic and pathogenic factors to explain this phenomenon, and whether this may be only a local inner city phenomenon or represents a national trend.

The IAIDP survey of medical schools in the U.S. to ascertain academic, clinical and teaching needs in allergy and clinical immunology has been analyzed. A 100% response of the 124 schools queried indicated deficiencies. In allergy, 69 (56%) departments of internal medicine reported unsatisfactory conditions, 59 without full-time staffs; 52 (42%) departments of pediatrics indicated unsatisfactory situations, 60 without full-time staff. In clinical immunology, 54 (44%) departments of internal medicine indicated unsatisfactory conditions, 38 without full-time staff; 60 (48%) department of pediatrics indicated unsatisfactory situations, 55% without full-time staff. Additionally, 14 departments of internal medicine and 18 departments of pediatrics were without part-time/salaried faculty in either allergy or clinical immunology. A report has been submitted for publication.

The ACIB has continued efforts to develop and implement cooperative endeavors in outreach and community activities by NIAID Asthma, Allergic and Immunologic Disease Centers (AADC and CIRID).

The Kidney Transplant Histocompatibility Studies (KTHS) has entered the second phase of analysis which consists of indepth evaluation of factors that have been identified as significant in phase one. Included in these are the effect of race of the recipient, perioperative and preoperative blood transfusions, and of diabetes as a primary disease. Another very important component of the second phase of analysis is the development of a statistical model which may be used to predict the outcome of transplants in individual patients. With the use of this model it should be possible to select recipients and methods of treatment to optimize the probability of success. This statistical model has been evaluated against the KTHS data base and also that of the Southeast Organ Procurement Foundation (SEOPF).

A formal collaboration has been established between the GTBB and the Medical Information Service of the HCFA End Stage Renal Disease Program to evaluate data principally on transplantation contained within the files of this federal agency. This task has been substantially completed acquiring identification of a population upon whom enough information could be made available to perform the requisite analysis and definition of the date of failure of the graft. Graft and patient survival were determined as a function of age, race, sex, and primary disease. In addition similar analysis of patient survival was performed for those patients on dialysis who had not yet been transplanted. The analysis have been submitted to the Endstage Renal Disease Program and plans for further cooperative endeavor are in progress, e.g. a mathematical model to project medical and economic impacts of change in treatment strategies on the population with chronic renal failure is being developed.

10. Liaison and Outside Activities

IAIDP Staff have joined with the Asthma and Allergy Foundation of America (AAFA) and the Lupus Foundation of America (LFA) in developing and effecting joint endeavors for educational and community activities to further the causes served by these lay groups. Additionally, IAIDP staff members have been contributing to the work of professional and scientific societies and to the educational and service endeavors of medical schools and clinical institutions.

Dr. Cohen serves on the Board of Directors and the Medical Advisory Council of the LFA, is Executive Vice President of LFA, and with Dr. Sogn served on the AAFA Committee for Public Education.

Dr. Robert Goldstein serves on the faculty of the George Washington School of Medicine and the medical staff of the G.W.University Hospital, Dr. Dorothy D. Sogn on the staffs of the Allergy Clinics at Walter Reed Army Medical Center and the NIH Occupational Health Unit, and Drs. Cohen, Goldstein and Sogn on the NIAID LCI Clinical Center staff.

Lectures to LFA Chapters were presented by Dr. Sheldon Cohen at Providence, R.I. in November, 1981, at Boston in May, 1982; at San Jose in June 1982; and to the LFA annual National Council of Representatives meeting in Buffalo during July, 1982.

Lectures to AAFA Chapters were given by Dr. Sheldon Cohen in December, 1981, in Knoxville and in February, 1982 at Los Angeles and by Dr. Goldstein in November, 1981 at Hollywood, Florida, .

Lectures to Professional Groups:

Dr. Sogn: at the American Academy of Allergy in Montreal, March 1982, on Penicillin Hypersensitivity; the New York Botanical Gardens, October 1981, on Tobacco Allergy; the Luzerne County (Pennsylvania) Medical Society, October 1981, on Penicillin Allergy; and the Fairfax Nurses' Association, October 1981, on Asthma and Allergy.

Dr. Goldstein: at Pediatric Grand Rounds, Medical College of Virginia, Richmond, November 1981; at the American Academy of Allergy, Symposium on Aspergillosis, Montreal, March 1982; at Society for Investigative Dermatology in April, 1982 on research growth and NIH; on Mold Allergies at the Eastern Pennsylvania Branch, ASM Medical Mycology Workshop in Philadelphia, June 29, 1982.

Dr. Krakauer: American Association of Clinical Histocompatibility Testing Panel on the Serum Bank April 25, 1982 and the American Society of Transplant Surgeons June 4, 1982.

Dr. Janicki: the American Association of Immunologists Committee on training affairs seminar on "A Primer for the Young Immunologist on the NIH Grant Process" at the Association's annual meeting New Orleans, April 1982, and similar seminars at the University of Mississippi Medical Center, Baylor College of Medicine, Columbia University College of Physicians and Surgeons, Tufts University School of Medicine, University of Zurich School of Medicine, Basel University, and Texas Southern University.

Dr. Cohen: seminar on "Eosinophils in Health and Disease" at the annual meeting of the American Academy of Allergy in Montreal, March 1982 and to the Mexican Society of Allergy in September, 1982 at Guadalajara.

Dr. Goldstein served as chairman of the Section of Allergy and Clinical Immunology of the American College of Chest Physicians and chaired three luncheon sessions at the College's Annual meeting in October 25-29, 1981 at San Francisco. The topics included were: "Immunologic Approaches to the Diagnosis of Drug Allergies", "Basic and Clinical Aspects of Eosinophils", and "Mast Cell Mediators and Asthma". In addition he co-chaired a symposium jointly sponsored with the Section of Occupational Lung Disease entitled Occupational and Environmental Allergic Lung Disorders. He served also as a panel member for a session entitled Pulmonary Infection in the Immuno-Deficient Host.

Dr. Goldstein served as a member of the Annual Meeting Program Committee for the American College of Allergists and chaired a session at the annual postgraduate course held in conjunction with the annual meeting January 17, 1982 at Bal Harbor, Florida. He completed his term as representative councilor for the Scientific Assembly on Microbiology Infection and Immunity of the American Thoracic Society, annual meeting Los Angeles, May 17, 1982.

Dr. Janicki chairs a trans-NIH Committee on Hybridoma Issues and has been involved with planning for a registry of hybridoma data. He represented the NIH group at an organizational meeting in Geneva under the sponsorship of the Committee on Data for Science and Technology of the International Council of Scientific Unions. As a result of this meeting, a CODATA Task Group is being formed to develop a computer-based hybridoma data bank to serve the international research and commercial communities. Dr. Janicki has been appointed as the U.S. representative to the Task Group with other members from France, Switzerland, Norway, England and Japan.

Dr. Dorothy Sogn has established liaison with the Council of the Graduate Department of Psychologists which held a meeting in Bethesda, February 10, 1982. Areas of mutual concern include "Defined Diets and Childhood Hyperactivity", the subject of an NIAID Consensus Conference, and self-help programs for asthma.

Dr. Cohen participated in a briefing on systemic lupus erythematosus for members of Congress and their staffs in January 21, 1982 in the Capitol.

Members of staff gave editorial support to the LFA in the development and publication of "Lupus Erythematosus: a Handbook for Physicians, Patients and their Families."

Under the direction of the ACIB, the AADC and CIRID Directors Workshop met on November 19-20, 1981. Outreach and demonstration programs, especially self-management of asthma programs were discussed and reviewed.

Dr. Krakauer organized a Symposium at the American Association for the Advancement of Science, January 3, 1982, in Washington D.C. entitled "Can we prevent organ grafts from failing? Studies on large populations as guides to policy and practice".

11. International Health Activities

Drs. Sheldon Cohen and Robert Goldstein serve on the International Union of Immunological Societies (IUIS)-World Health Organization (WHO) Committees: Dr. Cohen, the Committee on Standardization and Dr. Goldstein, as Secretary, the Sub-Committee on Standardization of Allergens. Dr. Goldstein attended subcommittee meetings in Frankfurt, Germany in October, 1981 and in Montreal, Canada in March, 1982.

Dr. Sheldon Cohen visited Geneva during February, 1982 as a consultant to the WHO Immunology Unit and participated in the work of the WHO-International Union Against Tuberculosis-Respiratory Diseases Committee in February, 1982, in London.

Drs. Sheldon Cohen, Dorothy Sogn, and Robert Goldstein have developed the questionnaire for the ongoing WHO epidemiologic study on the prevalence and nature of asthma and allergic disease in developing countries, initiated in Kuwait, Venezuela and Thailand.

Dr. Cohen continues to serve as consultant to the Ministry of Public Health in Kuwait for the development of an allergy center for diagnostic referrals, training and research to serve Kuwait and the Arabian Gulf area. As an initial step, an instructional course, with visiting faculty from the U.S., was developed for physicians selected to staff the Center on its anticipated opening in September, 1982.

The second joint meeting of the U.S. and Japanese Immunology Boards occurred May 12, 1982 in Bethesda, MD. At this meeting, the Boards reviewed the Guidelines planned for the next meeting to be held in conjunction with the Fifth International Congress of Immunology in Japan, and considered the distribution of hybridoma cell lines and other immunologic reagents and materials to facilitate research in both countries. Also identified as a worthwhile activity of this nature is the development of a registry, data base, or bank for the cDNA probes and genomic clones that are proving to be exceptionally valuable for ongoing studies of the immune system at the genetic level. A symposium entitled "Application of Recent Advances in Fundamental Immunology to the Study of Human Immune Response "was held simultaneously.

U.S. -People's Republic of China Program for Cooperation in the Science and Technology of Medicine and Public Health: IAIDP has been assisting the NIAID Director, Dr. Richard Krause, in his responsibilities as chairman for the U.S. Section on Immunology within the Joint Health Committee of the Developing U.S.-PRC Program. Specific studies for cooperative research will include HLA tissue typing and disease associations, allergy, immunity to infections and specific problems in clinical immunology. Plans have been made for Dr. Cohen to lead a delegation of U.S. allergists and clinical immunologists to meet with Chinese counterparts in Peking and other Chinese cities. Discussions will be held with the objective of defining possible areas where mutually supportive and cooperative investigations in allergy can be pursued.

IAIDP resources materials and histocompatibility sera have been provided to laboratories in foreign countries including Switzerland, France, West Germany, Netherlands, Russia, China, and Japan (Sections 5 and 6).

Drs. Sheldon Cohen and Dorothy Sogn at the request of the WHO have revised and updated the nomenclature on allergy and related diseases for the International Classification of Diseases.

IAIDP has continued to assist WHO in the planning and conduct of its month-long (September) annual course on Immunology of Infection for immunologists from developing countries. In addition to NIAID's support of the WHO Immunology Unit for this year's endeavor in Lausanne, Dr. Dorothy Sogn participated as a faculty member responsible for presentations on allergy and hypersensitivity manifestations of infection.

12. Publications

The following publications are concerned with IAIDP related work, projects and programmatic activities.

Krakauer, H.: Central Banking of Hybridomas. In Vitro, 17:1081, 1981.

Krakauer, H., Hartzman, R.H., and Johnson, A.H.: Monoclonal antibodies specific for human polymorphic cell surface antigens, I. Evaluation of methodologies. Report on a Workshop. Human Immunology, 4:167, 1982.

Krakauer, H. and Van Wyke, K.: Monoclonal antibodies in research on immunology and infectious diseases. Infectious Disease, Vol. 11, No. 12, page 4FF, 1981.

Krakauer, H., and Craney, C.L.: Solid state logarithmic amplifier for the D U spectrophotometer. Chemical, Biomedical and Environmental Instrumentation, 12:XXX, 1982.

Goldstein, R.A.: Allergy and Immunology in the 80's. Cardiopulmonary Medicine, October, 1981.

Goldstein, R.A., and Patterson, R.: Symposium on Occupational Immunologic Lung Disease, Annals of Internal Medicine, July, 1982, in press.

Goldstein, R.A., and Patterson, R.: Symposium on Occupational Immunologic Lung Disease. Journal of Allergy and Clinical Immunology, 70:1-72, 1982.

Rohatzi, P.K., and Goldstein, P.A.: Immunology of Sarcoidosis, Clinical Immunology Newsletter, August 1982, in press.

Mullally, D.I., and Sogn, D.: Manpower Needs in Allergy and Clinical Immunology, submitted for publication.

Mullally, D.I., Howard, W.A. and Hubbard, T.: Changing patterns in childhood asthma-A study in one pediatric hospital population, submitted for publication.

Schwartzman, R.M., Massicot, J.G., Sogn, D.D., and Cohen, S.G.: The atopic dog model-Report of an attempt to establish a colony, submitted for publication.

The GTBB catalog supplement for users of histocompatibility testing reagents, including homozygous typing cells, and the Manual of Tissue Typing Techniques (revised) continue to be made available for distribution.

The ACIB has prepared and distributed a catalog of available reference standards and reagents for investigations in allergy and clinical immunology entitled "NIAID Research Resources for Allergic and Immunologic Studies". It is planned to incorporate materials in this publication with that of comparable information available from the Food and Drug Administration and produce a NIAID-FDA combined resource catalog by collaborative effort.

A "Monograph on Adverse Reactions to Foods" is being jointly prepared by the NIAID and the American Academy of Allergy (AAA) Research Council Committee on Adverse Reactions to Foods. It will be co-edited by Dr. John Anderson of the AAA and Dr. Dorothy Sogn of our program. The Monograph which is to include sections on all types of adverse reactions will emphasize those of known or suspected immune and/or allergic origin. Now in draft stage, it is hoped that the publication will go forth in time for the March 1983 American Academy of Allergy meeting.

Based upon published material in the NIAID report of the Task Force on Asthma and the Other Allergic Diseases, Program staff has worked with the American Academy of Allergy Scientific and Educational Council in the development of material for a "Primer in Allergy" scheduled for publication in the Journal of the American Medical Association.

A cooperative effort with the LFA has resulted in the publication of a "Handbook for the Lupus (SLE) Patient."

A summary version of the Proceedings of the Joint NIAID-NHLBI Workshop on Basic and Clinical Aspects of Granulomatous Disease has been accepted for publication in Clinical Immunology and Immunopathology.

13. Conferences

During FY 1982 the following meetings, conferences and workshops relevant to program activities and functions were conducted by the IAIDP.

October 14, 1981.	Family Conference in Asthma and Allergies, NIH, Bethesda.
November 19-20, 1981	Asthma and Allergies: AADC and CIRID Directors meeting. Theme: Self-Management of Asthma and Outreach Community Activity and Demonstration Programs relevant to Asthma, Allergy and Immunologic Diseases, NIH, Bethesda.
January 3, 1982	In conjunction with the AAAS, a symposium was held entitled: "Can we prevent organ grafts from failing? Studies on large populations as guides to policy and practice." Washington, DC.
May, 1982.	U.S.-Japan Symposium "Application of Recent Advances in Fundamental Immunology to the Study of the Human Immune Response."

Additionally IAIDP program support was given to the following national and international conference and workshop endeavors.

January 13-15, 1982	Conference on Immunoglobulin D: Structure and function New York, N.Y.
February 8-12, 1982	9th International RES Congress, Davos, Switzerland.
May, 1982	Workshop on Macrophage Activation, Hilton Head, S.C.
July 5-9, 1982	Conference on Mononuclear Cells and Antibody Networks, Saxton's River, VT.
August 1-5, 1982	Third International Lymphokine Workshop, Philadelphia, PA.
August 23-27, 1982	9th Congress of the International Transplantation Society, Brighton, England.
August 28-31, 1982	Fifth Ir Gene Workshop, St. Louis, MO.
Sept. 12-15, 1982	Workshop on Primary Immunodeficiency Disease, Eastsound, WA.

Consensus Development Conferences

A consensus development conference entitled "Defined Diets in Childhood Hyperactivity" was held at the National Institutes of Health on January 13, 14, and 15, 1982. Childhood hyperactivity offically termed the "attention deficit disorder with hyperactivity", is an important health concern, the traditional therapy for which has included behavior modification, educational techniques, psychotherapy, and pharmacotherapy. A 1973 report by Feingold on clinical observations of the benefit of a diet free of salicylates and food additives on hyperactivity focused attention on diet modification as a new treatment approach. Apparent discrepancies between some clinical impressions of efficacy and much of the available scientific evidence prompted the scheduling of this consensus development conference.

On the first two days of the meeting experts presented evidence to a consensus development panel and members of the audience concerning some key questions surrounding use and efficacy of the diet. Panel members, drawn from the lay public, biomedical research, pediatrics, nutrition, immunology and allergy, clinical pharmacy, psychiatry and behavorial sciences, genetics, education, epidemiology, biostatistics, environmental health, and law considered the presented evidence and reached consensus on the issues presented. In summary, the panel believes that the defined diets should not be universally used in the treatment of childhood hyperactivity at this time. However, the panel recognizes that initiation of a trial of dietary treatment or continuation of a diet in patients whose families and physicians perceived benefits, may be warranted. Defined diets should not be initiated until thorough and appropriate evaluation of the children and their families and full consideration of all traditional therapeutic options has taken place. The panel went on to identify a number of critical gaps in knowledge which affect interpretation of the results of dietary intervention in the management of hyperactivity syndrome and suggested directions for further research in this field.

On February 25, 1982, the Asthma and Allergic Disease Centers Program, in cooperation with the American Academy of Allergy sponsored a State-of-the-Art Symposium on Occupational Immunologic Lung Disease. The purpose of the symposium was threefold: first, to bring together basic and clinical scientists for the exchange of information and ideas concerning this vital topic; second, to provide a program which would enable other individuals from both government and industry to obtain a comprehensive summary update on the state-of-the-art of occupational immunologic lung disease; and third, to develop a resource document on this problem.

14. Budget

The following shows the distribution of support by award mechanism for the activities of the Program during FY 1982.

IMMUNOLOGY, ALLERGIC AND IMMUNOLOGIC DISEASES PROGRAM

FY 1982 AWARDS

Award Mechanism	Number	Amount
Research Project Grants	517	$61,016,000
Research Centers	21	4,144,000
Research Career/Program Awards	44	1,757,000
Other Research Grants	14	290,000
Subtotal, Research Grants	593	$67,207,000
Research Training		
Individual Awards	40	741,000
Institutional Awards	27	2,429,000
Subtotal, Training	67	3,170,000
Research and Development Contracts	22	1,348,000
TOTAL		$71,725,000

ALLERGY AND CLINICAL IMMUNOLOGY BRANCH

I. Administrative Summary

A. Scope

This Branch is concerned with the etiology, pathogenesis, diagnosis, prevention and treatment of allergic and immunologic diseases.

Studies of allergic diseases supported by the Branch include: (1) factors which contribute to asthma, such as extrinsic allergens, infections, abnormalities of the sympathetic nervous system, chemical mediators of inflammation, and pharmacologic agents; (2) immediate type hypersensitivity and its disorders (allergic rhinitis, atopic dermatitis, urticaria and angioedema); (3) allergic phenomena affecting respiratory, gastrointestinal and cutaneous tissues; (4) allergic disorders caused by insect bites and stings, foods, airborne allergens, and infectious agents; (5) immunoglobulins, particularly IgE, and the chemical mediators released by the interaction of antigen and antibody on target cells; (6) therapy and prevention of allergic disorders and hypersensitivity reactions; (7) manifestations of delayed hypersensitivity and contact dermatitis; (8) mechanisms of drug reactions and chemical sensitization; (9) isolation and chemical characterization of the active fractions of known allergenic agents; and (10) epidemiologic and environmental studies designed to ascertain those agents or substances which may be of clinical relevance for allergic individuals.

In the area of immunologic diseases, the Branch activities are focused on studies of the underlying mechanisms of disease and the application of basic knowledge to the etiology, prevention and management of immunologic disorders. Studies in clinical immunology are directed toward acquired and inherited diseases associated with dysfunctions of the immune system. Immunopathology studies include genetics, cytology, biochemistry, physiology and pharmacology of the immune system. Areas supported by the Branch include studies of (1) immune deficiency diseases arising from primary defects in development or disorders affecting immune responses, (2) clinical manifestations mediated by products of lymphocytes, (3) diseases associated with immune complexes and autoimmune phenomena, (4) immunodermatology, i.e., immune disorders involving the cutaneous system, and (5) immunotherapy of disease processes.

B. Organization

Research grant activities supported within this Branch are broadly grouped under the general programs for Allergy and Clinical Immunology. In addition, this Branch supports a Program of Institute Emphasis (PIE) for Asthma and Allergic Disease Centers, Centers for Interdisciplinary Research in Immunologic Diseases, and Program Projects in Mechanisms of Immunologic Diseases. Finally, the Branch also supports a contract activity which provides reference and research reagents and materials to facilitate ongoing work.

1. Allergy

This program is concerned with investigations of the etiologic factors, pathogenetic mechanisms, diagnostic measures, and therapeutic approaches related to the effective management of allergic disorders. In addition, the Program on

Asthma is structured to investigate all of the major parameters contributing to or describing the mechanisms of causation in this disease. These include the natural history, study of allergens, bronchoprovocation challenge, animal models, pulmonary function testing, study of chemical and cell mediators, adrenergic agonists and antagonists, parasympathomimetic drugs, drug allergy, diagnostic methodology and therapeutic agents.

2. Clinical Immunology

Research grouped into this program category focuses broadly on combining and coordinating basic studies of the immune response with potential clinical relevance and application. The Program on Immunodeficiency, for example, is concerned with the etiology, ontongeny, prevention, and treatment of structural and functional deficiencies of the immune system, both naturally occurring and acquired disease states are included. Ongoing studies have focused on the congenital absence, failure of development, or other disorders affecting thymus or bone marrow cellular elements; abnormalities in production, inhibition in metabolism of immunoglobulins; deficiencies of specific complement components; and defective host defenses due to abnormalities of leukocytes. These naturally occurring or acquired defects of immunity provide unique opportunities for scientists to expand our understanding of normal immune functions. Although some of these disorders are rare, the information gained by studying them is relevant to the general field of immunology and to many health problems including those in infectious diseases, allergy and arthritis.

Studies focused on immune complex disorders aim to clarify the role that immune complexes play in the production of disease. Immune complexes which result from the binding of antigens by antibodies and arise during the course of development of the normal immune response. Under pathologic circumstances, they may appear within and harm organs and tissues. The mechanisms by which immune complexes cause tissue damage, particularly in the autoimmune diseases with which they are increasingly associated, is a subject of intense investigation.

Similarly studies of inflammation, which is mediated by cells and humoral factors, are essential for our understanding of clinical immunology. While the process of inflammation is required for host defense against foreign substances, in certain disease states inflammation plays a major role in causing tissue damage. An understanding of what initiates and therefore what modulates inflammation is crucial to understanding and controlling these disease states. The noncellular mediators of inflammation can be divided into two general categories, those of low and those of high molecular weight. The group of serum proteins derived from the complement system and the associated enzyme systems of coagulation fibrinolyces, and kinin fall into the latter category. The initiation of the complement cascade by either the classic or alternate pathway makes a major contribution to the development of the inflammatory response. Once activated, complement components have functions including opsonization, immune adherence, chemotaxis, cell lysis, and anaphylotoxin generation. Derangement of complement have been implicated as causing or contributing to a wide variety of disorders including cancer, kidney diseases, collagen vascular disorders, infections diseases, and inherited diseases.

3. Program of Institute Emphasis for Asthma and Allergic Disease Centers Program (AADC)

This Program currently supports a network of 18 Asthma and Allergic Disease Centers, located throughout the United States, and is actively engaged in both fundamental and applied research. Through this multifaceted approach, methods have already been developed to improve the diagnosis and treatment of asthma and other allergic diseases. Although the emphasis of the Centers Program is directed towarded a better understanding of the basic mechanisms involved in the various allergic disorders, they also have provided the field of allergy with unique academic resources for the discipline of allergy. By serving as referral centers for patients in their areas, engaging in collaborative clinical trials to assess diagnostic and therapeutic discoveries, and acting as an important resource for training academic allergists for the future, they have had a profound impact on the delivery of health care to allergic individuals. Separately each of these Centers has also been successful in competing for additional support through either the Regular Grant Program, Training Grants, or Research Career Awards.

This Program of Institute Emphasis represents a major financial and philosophic commitment by the IAIDP and the NIAID to the elucidation of allergic disorders. In addition to providing support for basic science, periodic working group meetings of the Center Directors have been held, usually on an annual basis. These meetings foster continued scientific exchange as well as encourage efforts to ensure outreach activities, explore mutually beneficial community programs and other teaching methods along with establishing a network of communication among Centers, the NIAID and communities nationwide.

Renewal and new applications for this program are received on an annual basis in an open competition.

4. Program of Institute Emphasis for Centers for Interdisciplinary Research in Immunologic Diseases (CIRID)

In September of 1978, four Centers for Interdisciplinary Research in Immunologic Diseases were funded by NIAID. This Program is designed to foster integration and coordination of research projects in Clinical Immunology being pursued in clinical specialties (for example, dermatology, pulmonary medicine, hematology, nephrology, rheumatology, infectious diseases and otorhinolaryngology) with those in basic research (for example, immunochemistry, microbiology, virology, genetics, biochemistry, pharmacology, general physiology, and pathology). Furthermore, study of one or more allergic diseases is a necessary component.

An important additional component of these Centers is the funding of specific projects concerned with a variety of community activities which are designed essentially to impact on health care in a tangible and immediate way. It is hoped that these efforts along with the traditional basic research components may provide guidance and future direction on how to best accomplish this transfer of technology.

During FY 1983 the original four Centers will be competing for renewal along with new applicants for continuation of this Program.

5. Program of Institute Emphasis in Mechanisms of Immunologic Diseases

Program Projects concerned with Mechanisms of Immunologic Diseases were established to encourage the development of collaborative basic science and clinical investigation in immunologic diseases. As such, this program is designed to further investigate underlying mechanisms of disease and to enhance basic knowledge relevant to etiology, prevention and management of immunologic disorders. Clinical immunology studies are directed toward acquired and inherited diseases associated with dysfunctions of the immune system. Immunopathology studies includes specific areas of genetics, cytology, biochemistry, physiology, and pharmacology of the immune system and its disorders.

This Program currently funds seven Program Projects and on an annual basis renewal applicants compete with new applicants. While there is now requirement that these Program Projects include studies of allergic disorders, collaboration is encouraged among Asthma Centers, CIRIDs and Program Projects in Mechanisms of Immunologic Disorders by periodically convening working group meetings among the directors and key individual investigators.

6. Research Reference Reagents

The Contract Mechanism has been employed by this Program to assist current research with an emphasis on studies of allergic disorders by providing useful and unique reagents and materials to investigators. In order to aid in this process a Resource and Reference Laboratory whose function is the acquisition, quality control testing and distribution of important and unique reagents and materials has been established at the Mayo Clinic.

C. Awards and Support Levels

Relevant activities in Allergy and Clinical Immunology are supported through various mechanisms including contracts, individual post-doctoral fellowships, institutional pre- and post-doctoral training grants, career and career development awards, as well as investigator initiated research grants.

The following shows the distribution of support by award mechanisms for the activities of the Branch during FY 1982. (Continued on page 3-5)

Allergy and Clinical Immunology Branch
FY 1982 Awards

Award Mechanism	Number	Amount*
Research Grants	213	$ 24,161,080
Career and Career Development Awards	15	$ 598,146
Subtotal	228	$ 24,759,226
Fellowship Awards	9	$ 160,577
Training Grants	21	$ 1,763,337
Subtotal	30	$ 1,923,914
Contracts	3	$ 137,000
Total	261	$ 26,820,140

*The statement for the PIE activities is included in the Research Grant Category. During FY 1982 the Branch Supported these activities with awards for 18 Asthma and Allergic Disease Centers at a total cost of $4,216,330, seven Clinical Immunology and Immunopathogy Program Projects at a total cost of $3,985,227, and four Centers for Interdisciplinary Research in Immunologic Diseases at a total cost of $1,290,022.

II. SCIENTIFIC SUMMARY

1. Allergy

The discovery of serum IgE has helped the practicing physician to better understand the wide spectrum of symptoms presented by allergic individuals. In addition, studies have led to better means of diagnosis and treatment of allergic rhinitis, asthma, eczema, urticaria, and other disorders. Basic studies aimed at the elucidation of mechanisms of the IgE mediated immediate type hypersensitivity reactions have shown that specific IgG blocking antibodies may be formed as a consequence of immunotherapy. In selected instances these antibodies actually prevent the occurrence of reactions between allergen and IgE bound mast cells. Moreover, the IgE antibody response has now been shown to be regulated both by cellular, (suppressor T-cells) as well as humoral factors (enhancing and suppressor). Atopic individuals have also been shown to generate less histamine induced suppressor T-cell activity than non-atopic individuals. Other studies aimed at elucidating the regulation of IgE synthesis have shown a severe deficiency in suppressor T-cells in certain individuals with hyperimmunogobulin-E. syndrome a severe deficiency in suppressor T-cells. Additional work has demonstrated that by chemically altering individual allergen preparations which can then be used for immunotherapy it is possible to retain clinical efficacy, reduce side effects and induce immunologic tolerance. Thus studies of polymerized ragweed antigen preparations have confirmed the potential clinical importance of these extracts.

By taking advantage of this basic knowledge concerned with the immunoregulation of immediate type hypersensitivity reactions it should be possible in the near future to develop specific or non-specific "factors" which are capable of regulating allergic reactions either "up" or "down" depending upon the desired clinical outcome. One hope is that some of these discoveries may make classical immunotherapy become an obsolete form of treatment.

Similar excitement has been generated by studies aimed at clarifying the role of chemical mediators in allergic reactions. Most studies of mediators have examined the role of histamine in allergic reactions, a preformed mediator which is released from mast cells during allergic reactions. It is for this reason that antihistaminic compounds are used for the treatment of these reactions. Until recently, however, much less was understood about the roles those molecules which were produced after IgE mediated events. It is now known that derivatives of arachidonic acid, a fatty acid component of membrane phospholipids, appear to play a key role as chemical mediators in either causing or promoting allergic reactions. Some of these arachadonic acid metabolites include prostaglandins, which have been shown to demonstrate opposing effects on central airways and vessels; slow reacting substances of anaphylaxis (SRSA), now called leukotrienes, which have been shown to cause bronchoconstriction, and more recently acetylglyceryletherphosphorylcholine (AGEPC), formerly called platelet activating factor, which has been demonstrated in small quantities to produce anaphylaxis in animals.

The leukotrienes, which have been chemically characterized only within the past few years, are 600-9000 times as potent as histamines in causing itching, nasal iritation and swelling during allergic reactions. Leukotrienes have been demonstrated to also constrict small airways, which may be a cause of breathing difficulty in asthma, and may increase permeability of small blood vessels, constrict coronary arteries and cause white blood cells to concentrate at inflammatory sites. Human studies have now for the first time supported the laboratory findings for a role of leukotrienes in asthma and it has been shown that they were extremely potent in reducing expiratory volume of small airways in healthy volunteers. Thus the recent discoveries of both the chemical structures of the leukotrienes as well as AGEPC have made it possible for investigators to fully explore the mechanisms by which these substances actually induce bronchoconstriction and changes in vascular permeability. Exploitation of these discoveries in the next few years should make it possible to develop specific antagonists, agents which can pharmacologically intervene at precise pathways of allergic inflammation acting to abrogate the clinical response. Moreover, it should be possible to accomplish this prior to the definition and clarification of the particular inciting agent.

2. Clinical Immunology

Among one of the more important mechanisms essential to our understanding of how inflammatory and immunologic reactions occur is the complement system. It was originally defined as those factors present in fresh serum thought responsible for the lysis of antibody coated (sensitized) bacteria and cells. It is now known that in addition to lysis the function of this extracellular effector pathway includes the mediation of opsonization and the induction and modulation of other pathways of inflammation. Activation of the complement system is often induced by antigen antibody reactions and the activation process appears to be an important concomitant of the host defense reaction leading to the destruction of, for example, micro-organisms. In addition, complement activation leads to the generation of factors that can mediate membrane damage of cells. This capacity to damage tissue accounts for the very important role of complement in many of the disorders that have an immunologic basis.

It is important to emphasize the variety of biologic functions that result from activation of the complement system these including the enhancement of phagocytosis (opsonization) and viral neutralization and mediators of inflammation; cell lysis; and modulation of the immune response.

Given the wide variety of potential biologic functions and activities it is not surprising that complement levels have been measured in a wide variety of diseases. The most common finding, especially in infectious and inflammatory disorders is elevation of complement titers. In these situations, complement behaves as an acute phase reactant. The CH-50, C-4, C-2, C-3, factor B, C-1 and C-3 inhibitors as well as additional components are increased sometimes as much as two or three fold. Increased synthesis probably accounts for this finding. Another common alteration in complement levels occurs in criticaly ill individuals who may manifest disseminated intravascular coagulation. In these disorders C-50 and C-3 have been found to be variably depressed. In most patients with hereditary angiodema, systemic lupus erythematosus (SLE), complement mediated hemolytic anemias, and a number of glomerulonephritides as well as renal transplant recipients, increased catabolic rates have been demonstrated accounting for reduced serum concentrations. On the other hand in a few patients with SLE and many with hypocomplementemic membranous glomerulo nepheitis reduced synthesis has been demonstrated. In summary, in most inflammatory disorders, the increased complement serum levels observed are secondary to enhanced synthesis, while the the decreased serum concentrations observed in certain deseases associated with antigen antibody interactions are caused by augmented degradation and complement activation.

There are two general types of assay procedures in use. The first of these measures the level of the various complement proteins as antigens in serum by simple immunoprecipitation techniques. The second measures the activity of individual components or groups of components by functional assays. Our enhanced ability to accurately measure components of complement in a variety of diseases has led to widespread use of these measures where appropriate. Generally speaking, the complement proteins circulate in an inactive precursor state. Activation of complement then leads to the utilization of the components and a fall in the level in the blood. Typical patterns seen in clinical disorders would include generalized hypocomplementemia in SLE, serum sickness, cryoglobulinemia, as well as endotoxemia and occasionally immune complex mediated disorders.

Generally speaking, diseases of the complement system can be divided into two groups. The most important comprises those illness in which complement is functioning normally, but is being activated in inappropriate tissue sites or under inappropriate circumstances. Thus, the normal activation of complement by antibody coated erythrocytes may lead to hemolytic anemia; antibody to renal glomerular antigens may activate complement leading to Goodpasture°s disease, etc. In these situations the complement system is acting normally to produce inflammation and cell damage. The second group of diseases are the deficiency states, which are quite uncommon. Here failure to senthesize a particular component leads to an interruption of one part of the activation sequence. The primary importance of these disorders is that they provide insight into the normal biology of the complement system. Clinical examples of the former include studies performed in SLE, which has probably been examined in greater detail than other disorders. Approximately two thirds of patients with active lupus have reduced complement concentrations and practically all patients with multisystem disease, renal involvement and positive anti-DNA antibodies have reduced C-4 and C-3 levels. In lupus, antibodies are formed against a variety of serum tissue constituents, especially components of the nucleus. These nuclear antigens and antinuclear antibodies as well as other antigen antibodies systems form immune complexes that combine with C-1Q and activate the classic complement pathway. Furthermore, it may demonstrated by immunoflurescence that antigen antibody complement complexes are deposited in the glomerulus, epidermal-dermal junction of the skin, central nervous system and other organs. It is not surprising that serum levels of C-1Q, C-4, C-2, C-3, and other late components such as properdin and properdin factor B maybe depressed in lupus.

A notable example of the lack of an inhibitor is the clinical disorder of hereditary angioedema, which is the most common genetically determined disease of the complement system and is caused by reduced functional activity of the C-1 inhibitor. The defect itself is transmitted as an autosomal dominant, patients have characteristic episodes of nonpainful, nonerythematous, nonpruritic, non pitting edema involving most commonly extremities, GI tract and airways. The attacks are often precipitated by mild trauma, last two to four days, do not respond to the usual therapy, and disappear without sequelae unless airways are involved. Diagnosis of the disease rests on the typical clinical history and demonstration of reduced levels of C-1 inhibitor as well as C-4. Current methods of treatment involve replenishing that inhibitor deficiency either by drugs or blood products. Information gleaned from the studies of the components, the basic mechanisms, and the enzyme deficiencies will hopefully provide means to enhance the beneficial reactions and diminish the harmful effects of complement activation during the course of inflammatory and immune responses.

It is not coincidental that many immune complex disorders are closely interrelated with our understanding of the complement system. Our current knowledge of the functioning of immune complexes in the development of disease states began with the realization that antibody antigen reactions in the circulation did not always lead to beneficial results. The classical experiment stemmed from observations made during a careful analysis of the results of infusion of horse, antidiptheria antitoxin into man and of the consequent development of serum sickness in some patients. After those initial observations, three models of antibody mediated tissue damage were developed which have become representative of a number of types of immunologically mediated diseases in man. These include a model for anaphalaxis, Forssman shock and the Arthus reaction. The importance of these different mechanisms of allergic or hypersensitivity reactions in man varies widely. Local anaphylactic responses in the respiratory mucosa are responsible for most respiratory allergies. Because of the requirement for high local concentrations of antigen and antibody, Arthus reactions are relatively uncommon, but can occur as an integral part of a mechanism of allergic tissue damage in the lung in the case of allergic alveolitis. Allergic reactions involving cell bound antigens can occur during the transfusion of blood, owing either to the presence in the recipient of antibodies to the transfused cells or to the transfusion of antibodies that react with the cells of the recipient. The analogous clinical situation to the Forssman reaction is Goodpasture's syndrome in which antibody to a tissue component, the glomerular basement membrane is fixed, activates complement and causes structural tissue damage.

The most well developed and carefully studied model of immune complex mediated tissue damage is that of experimental serum sickness and glomerulo nephritis in the rabbit. In the first model, acute serum sickness, a single injection of a large quantity of heterologous serum protein, such as, bovine serum albumin is injected intravenously into the rabbit. In another model, serum sickness is elicited in the rabbit by repeated injection of the heterologous protein.

Using these models factors that govern the deposition of immune complexes in glomeruli and blood vessels have been closely studied. Thus presence of vasoactive amines such as histamines or serotonin leads to separation of endothelial cells along the wall of blood vessels, with subsequent leakage of immune complexes from the circulation into the vessel wall. It is believed that initial steps in the leakage of immune complexes into glomerular or other vessel walls in man may also involve the action of vasoactive amines such as histamine, perhaps mediated by the action of IgE antibodies and mast cells. The experimental importance of studying the mechanisms of formation and clearance of immune complexes cannot be overemphasized. There are a wide variety of clinical manifestations that have been associated with circulating immune complexes, including vasculitis, glomerulonephritis, arthritis, dermatitis, pleuritis, shock, leukopenia, thrombocytopenia and others. The collagen vascular disorders have been most widely studied. Among these, studies of SLE disclose that kidney lesions in patients with lupus nephritis appear quite similar to those found in rabbits with acute serum sickness. Further support for the concept of the role of circulating immune complexes in lupus came from the observation that the presence in the serum of antibody to native DNA and low serum complement levels generally correlated with active lupus nephritis. More recent studies have demonstrated that patients with SLE have prolonged clearance of autologous red blood cells coated with IgG from the circulation suggesting impaired function of the reticular endothelial system (RES); in clearing these receptors. The prolongation of the clearance in these individuals was found to be correlated with levels of circulating immune complexes measured by the C-1Q binding assay and with the clinical disease activity. It does appear that patients with lupus have circulating immune complexes as well as defective function of the RES. In patients with rheumatoid arthritis immune complexes are demonstrable in the serum and synovial fluid, although it is uncertain as to whether they are directly related to the disease process or occur as a result. Enhanced understanding of why immune complexes form and deposit in tissues should lead to targeted therapeutic intervention.

The immune system has an intricate network of self-stimulating, self-regulating cellular receptors and signals. Macrophages present antigens to T-cells while helper and suppressor T-lymphocytes signal B-cells to make respectively more or less antibody. Also, some B-lymphocytes regulate the production of antibody by other B-cells via networks of anti-idiotype antibodies. Depending upon which signals predominate, the outcome is a state of immunity or tolerance to a specific antigen.

An understanding of how regulation of the immune system occurs normally will be very useful in the prevention, diagnosis, and treatment of immune deficiency disorders and immunological diseases due to aberrations in regulation. Studies in these areas are occurring on several levels including the cellular and molecular levels.

Among the most exciting techniques of the future is lymphocyte cloning. These clones could be employed to probe the association between certain diseases and transplantation antigens the knowledge of which could be used to determine that segment of the population at risk and allow intervention in or prevention of the disease process. Once cell-to-cell communication is understood, the potential exists to direct the immune response, either to overcome deficiences or suppress an inappropriate response such as occur in autoimmune disorders. Techniques such as microsequencing of proteins, DNA recombination and monoclonal antibody production will permit the isolation and dissection of the signal molecules.

In a similar vein, several new potential clinical approaches to immunosuppression are breaking on the horizon, total lymphoid irradiation (TLI), cytopheresis and monoclonal antibodies. These could be used in the treatment of transplantation recipients and patients with autoimmune and immune complex disorders. During recovery from TLI suppressor mechanisms appear to dominate. Also cytopheresis could be used to remove immunocompetent cells and monoclonal antibodies could be employed to deplete, selectively, specific helper cell subsets.

Asthma and Allergic Disease Centers

Several of our Asthma and Allergic Disease Centers have concentrated their investigations on studies aimed at elucidating allergic and immunologic mechanisms responsible for causing asthma and other lung disorders. Moreover, during the past decades it has been recognized that a large number of respiratory disorders in the workplace have an immunopathologic basis. These include asthma, bronchitis, interstitial fibrosis, and hypersensitivity pneumonitis. Work-related asthma appears to be especially common. In the United States it is estimated that as many as two percent of all cases of asthma are of occupational origin. In Japan about fifteen percent of cases of asthma in man are considered to have an industrial cause. The importance of these observations originally noted in the 1979 NIAID Task Force on Asthma and Other Allergic Diseases concluded that it should be possible to prevent certain of these occupational respiratory disorders given the proper diagnostic tools and application of appropriate measures in the workplace. As a consequence of these findings in February of 1982 a state-of-the-art symposium on Occupational Immunologic Lung Diseases was held in Washington and sponsored jointly by the NIAID and the American Academy of Allergy. The purpose was to bring together basic and clinical scientists to exchange current information, to enable persons from government and industry to obtain up-to-date knowledge and to collectively develop ways to implement the findings and recommendations.

As a consequence of this meeting, which was published in the July 1982 issue of the Journal of Allergy and Clinical Immunology, volumne 70-1, it was determined that recognition and prevention of occupational immunologic lung disease will depend upon team work. That the problems are often complex and difficult to define so that a single scientific discipline does not have sufficient breadth to recognize, diagnose and treat. A role is clearly defined for collaboration among occupational physicians, epidemiolgists, allergists and clinical immunologists, pulmonary physicians and scientists with expertise in disciplines such as basic chemistry and air sampling. A useful approach will need the collaboration of the many diversely interested parties, including government agencies, industry, academic institutions, consumer groups and labor organizations to successfully accomplish the task of preventing disease and promoting health among workers.

In this regard, several of our Center Directors in collaboration with the NIAID have taken the initiative to establish a liason working group broadly composed and aimed at applying this information.

CIRIDS

Among the accomplishments emanating from the CIRID Program, scientists have made progress on the development of an effective vaccine against Hemophilus influenza type-B, a bacterium that causes meningitis, arthritis, severe ear infections and pneumonia especially in children. Others have studied the long term consequences of severe virus infections on the airways and lungs during infancy. The data suggest that these infections plays a significant role in the later development of asthma. This finding provides a rationale for the development of relevant viral vaccines against lung damaging infections of infancy, and thus may reduce the incidence of childhood asthma. Additional studies have included the discovery of an agent that can decrease the cellular level of glutathione, a compound that plays a key role in immune functions. This observation hopefully can lead to a class of specific drugs for the treatment of immune disorders. Scientists at one of our CIRIDs were among the earliest to describe the series of clinical disorders now known to compromise the so-called acquired immunodeficiency syndrome (AIDS) when they discovered a virtual epidemic of an unusual pneumonia due to pneumocystis carinii occurring in thegay community. Alarming numbers of cases of Pneumocystis carinii pneumonia and other serious unexplained opportunistic infections concentrated among but not exclusively affecting homosexual men have since been described throughout the United States. AIDS is associated with an underlying state of profound immunosuppression characterized by marked suppression of peripheral blood inducer/helper T-lymphocytes. Affected patients have very often presented with a symptom complex of chronic fever, weight loss and lymphadenopathy as a prodome to the development of either an opportunistic infection or Kaposi's Sarcoma (another almost uniformly lethal clinical manifestation of this syndrome complex).

At this time, several of our Centers, Program Projects and Individual Investigators are working intensely on clarification of this perplexing nationwide problem.

In addition, and because of the mandate for outreach demonstration programs the four CIRIDS have initiated lectures, workshops and seminars to educate and train primary care physicians and other health care professionals in basic immunology, as well as, the latest treatment techniques in asthma and allergic diseases. Other outreach programs have been directed at educating patients, their families and the general public in order to help those suffering from asthma to better help themselves. This latter program led to the convening of an important nationwide conference concerned with self-management educational programs for childhood asthma (conference summaries available upon request).

The Conference on Self-Management Educational Programs for Childhood Asthma, held June 11-12, 1981, in Los Angeles, California, brought together for the first time the developers of eleven major asthma self-help programs from across the United States, as well as a number of distinguished evaluators and interested observers.

In the past decade there has been a renaissance of scientific interest and research in health education and self-care, particularly as they apply to the management of chronic, disabling diseases. In recent years, a number of self help projects focused on childhood asthma have been developed and pilot tested in various communities across the nation. These programs, which evolved virtually independently of one another, were developed because physicians dealing with children with asthma felt that the care of these patients could be strenghthened and extended by organized approaches to dealing with the issues common to large numbers of patients. The Los Angeles conference was intended to enable researchers working in this area to share their progress, difficulties and ideas, to sharpen their understanding of the field and to facilitate and strengthen existing and new programs.

As a consequence of this meeting, NIAID and the Asthma and Allergy Foundation of America, in concert with, the CIRID Program and our Asthma and Allergic Disease Center Program are supporting continued studies aimed at further clarification and elucidation of more detailed and widespread programs concerned with self-help of childhood asthma as well as for adults.

Our network of Asthma and Allergic Disease Centers as well as CIRIDS, allows implementation of the findings from this continued effort to be expediously explored nationwide and represents another advantage of these programs.

Resource and Reference Laboratory

During FY 1982 establishment at the Mayo Clinic of a Resource and Reference Laboratory helped us acquire and assess new reagents and foster collaborative clinical trials among interested investigators was accomplished. Examples of some of this work which is in process or shortly coming to fruition include:

1. Planned acquisition of three International Reference Allergenic Extracts (in collaboration with the International Union of Immunologic Societies Subcommittee on Allergen Standardization of the World Health Organization) including short ragweed, mite and grass. Work is currently underway to expand this list for next year.

2. Availability, shortly, of a series of food extracts for both skin testing and oral challenge testing. These include milk, peanut, whole egg, wheat, soy bean, beef, shrimp, rice, chocolate and corn. It is our intent to distribute these materials to interested investigators in order to further explore the large clinical problem of appropriate and objective identification of allergies to food substances.

3. Dissemination of other materials to aid investigators including standard extracts to diagnose hypersensitivity pneumonitis (including aspergillus, pigeon breeder's, and farmer's lung). In addition and where appropriate we intend to explore collaborative studies of special diagnostic lab tests, and also clinical therapeutic studies of immunotherapy where relevant.

The importance of well characterized allergenic extracts for use in clinical studies can not be over-emphasized. Our aim is to utilize these materials for distribution to investigators in both our Asthma and Allergic Disease Center Programs, our CIRID Programs, and other Programs where they conduct those clinical studies which hopefully will elucidate and clarify mechanisms of allergic reactions and responses of individuals to immunotherapy with allergenic extracts. A listing of all materials and methods of obtaining them is available upon request from this office.

Genetics and Transplantation Biology Program

I. Administrative Summary

A. Scope

This Branch supports and manages research on the immunologic and genetic factors that determine the acceptance or rejection of grafts.

The increasing prevalence of degenerative disease and of trauma that may impair the function of particular organs sufficiently to be incapacitating or lifethreatening identifies the perfection of a successful transplantation methodology as one of the objectives of highest priority in current medical science. The technical surgical aspects have proved quite manageable but immunological rejection of the graft is the obstacle that has yet to be surmounted.

Research efforts supported by this Branch include:

1. Elucidation of the structure, organization and control of the expression of genes on which immunological activity depends.
2. Studies of strategies designed to minimize the likelihood of rejection by assuring the best possible antigenic match between donor and recipient.
3. Explorations of immunosuppressive regimens whose objective is to reduce the capacity of the host to reject the graft.
4. Studies of treatments of the graft that will reduce its ability to provoke a response.

Studies in animal models are supported that explore the genetic controls of the immune response, and, on a more practical level, test methodologies whose ultimate application is to man.

Genetically conditioned associations between antigenic characteristics and susceptibility to various diseases have recently been brought to light and also fall within the purview of this Branch.

B. Organization

Support for research in immunogenetics and transplantation immunology is provided principally through grants for investigator initiated proposals.

In addition, a significant research and development effort is funded through contracts, the preponderant portion of which is administered by the Serum Bank. It is an international resource, providing sera and homozygous typing cells for research on human immunogenetics and for histocompatibility testing performed in conjunction with organ transplantation. The continuing evolution of the Bank is currently taking it into information gathering and analysis in the field of population immunogenetics.

C. Awards and Support Levels

The Genetics and Transplantation Biology Branch supports research activities through individual grants, research career development awards, fellowships, training grants, and very importantly, in connection with its organ transplant program and serum banks, through contracts.

The following shows the distribution of support for the activities of the Branch during FY 1980.

Genetics and Transplantation Biology Branch

FY 1982 Award

Award Mechanism		Number	Amount*
Research Grants		85	$ 9,734,087
Career and Career Development Awards		10	327,021
	Subtotal	95	$10,061,108
Fellowship Awards		8	147,676
Training Awards		0	
	Subtotal	8	$ 147,676
Contracts		23	1,319,552
	Total	125	$11,528,336

* Total Costs, indirect cost estimated

The majority of these awards are concerned directly with transplantation biology and the remainder, a rapidly increasing component, support research on the genetics of the immune system in general and of susceptibility to disease, in particular. Approximately 30% of these awards were for competing new or renewal applications; the remainder represents commitments to support awards made in prior years.

II. Scientific Summary

The fields of immunogenetics and transplantation immunology have been greatly advanced in the past year as a result of the application of concepts and methodologies developed in molecular biology and basic immunology. The greatest ferment has occurred in investigations on man, but animal models continue to be used highly productively.

1. Molecular Genetics

Understanding of the organization and processing of genes and mRNA coding for immunoglobulin has reached a mature stage. Production of functional immunoglobulins is the end-result of an extended complex structural rearrangement of genes during the process of maturation of B lymphocytes, and also of the RNA transcripts. Although the heavy and light chains are encoded by chromosomally unlinked regions, the coding (exons) and non-coding (introns) regions of each chain are linked. During maturation and production of specific antibody, chromosomal rearrangements occur first in the regions coding for the variable portions of the heavy (V_H) and the light (V and V). These rearrangements involve splicing of one of the V sequences with a D segment and one of the J genes. The assembly is then spliced to one of the constant-region sequences, one for each isotype. There also occurs post-transcriptional processing, for example, leading to mRNA coding for membrane-bound or secreted IgM. The V-D-J splicing, because combinations of various V and J genes are possible and because of diversity at the V-D-J junctions, results in much antibody combining site diversity. In addition, somatic mutation in V_H further increases the diversity possible, although, in the case of at least the murine response to phosphoryl choline, it seems to affect the IgG and IgA but not the IgM isotypes. Efforts to count the number of V_H genes have led to estimates which depend on the method used and cover the range 40-2000. Such numbers, the joining mechanisms cited above, and combinatorial association of light and heavy chains can account for the size of the antibody repertoire.

Major efforts are currently underway in a number of laboratories to clarify the structure and expression of genes coding for class I (H-2K,D,L of mouse, HLA-A,B,C of man) and class II (H-2I of mouse, HLA-DR of man) antigens. The organization of H-2 like genes, consisting of several exons with intervening introns, has been determined, and a class I HLA gene has been sequenced. Multiple genes with sequences homologous to those coding for class I MHC antigens are found on chromosome 17 of mouse and 6 of man. Whether they all code for functional proteins and what the biological functions of the proteins not yet found by serological methods might be is not yet known. The extensive polymorphism of the MHC antigens could be the result of rearrangement analogous to those observed in the immunoglobulin genes. However, no such rearrangement has yet been found in the case of H-2I genes. On the contrary, there is extensive genomic polymorphism.

Analysis of the MHC genes has clearly not yet advanced to the point of permitting firm conclusion, but progress is extremely rapid.

Molecular genetic analysis of other structures with immunological function is also in progress. These include T cell suppressor factor and the T cell receptor.

2. Transplantation Immunology

Cell cloning has become a very important tool in many areas of immunology, including transplantation immunology. One objective of cell cloning is the production of abundant quantities of immunologically active substances which could be used to manipulate the immune system of transplant recipients and of persons with immunological disorders. Although the expansion and cloning of normal T and B cells has been achieved, the most efficient approach to the production of large quantities of secreted products requires the fusion of these cells to neoplastic, and therefore continuously proliferating, T and B cell lines. In this manner, monoclonal antibodies specific for functionally distinct lymphocyte subsets were developed and are currently in use both as diagnostic and therapeutic agents in transplantation. Currently under development and investigation are fused lines producing human suppressor substances. To date, T cells producing both non-specific and antigen-specific suppressors have been cloned, but only fusions resulting in the secretion of non-specific human suppressor substance have succeeded.

Antigen-specific feed-back regulation can be effected not only by means of antigen-specific suppressor substance but also by means of antiidiotype antibody. Here too substantial progress has been made in human systems. The antibodies of greatest interest are the ones recognizing receptors for HLA antigens because of the role the HLA antigens play in restricting the immune response and in organ rejection. Indeed, a cellular response (MLC) has been obtained in which the stimulant is a lymphocyte which specifically recognizes a DR antigen (DR2) and presumably bears the anti DR2 idiotype. Use of the MLC responder blasts for in-vitro immunization of human lymphocytes followed by fusion with a human fusion line should result in production of monoclonal human antiidiotype antibody. A more direct approach, examination of lymphocytes of multiparous women who have at one time produced but have ceased to produce anti-HLA antibodies, has revealed an active antiidiotype response causing the loss of the anti-HLA activity. The antiidiotype B cells in such cases can also be used for fusion and production of monoclonal antibody. Such antibodies will clearly be highly useful in investigations of the biologic role of MHC antigens, and should, if the logistics proves to be not too forbidding, along with suppressor factors usher in a new era of immunologic manipulation.

A very considerable body of information has been acquired in the past year on the detailed effects of immunosuppressive strategies on the functionally distinct lymphocyte subpopulations. Cyclophosphamide at low doses preferentially decreases the numbers of B cells in the circulation, followed by T suppressor cells as the dose increases, and finally becomes non-selective. Treatment of patients with anti-thymocyte globulin eliminates all lymphocytes. However, during the recovery phase, there is initially a predominance of the T suppressor/cytotoxic subset. Intravenous methylprednisolone transiently selectively reduces levels of helper T cells in the circulation. Total lymphoid irradiation also results in a predominance of the T suppressor/cytotoxic subpopulation. The implications of these findings for immunosuppressive strategies, i.e. the use of monitoring of lymphocyte subsets to guide immunosuppression, are currently being evaluated in pilot trials. Also currently in progress are clinical trials of subset-specific monoclonal antibodies as immunosuppressants.

3. Polymorphic Cell Surface Antigens

As described above, research on cell surface antigens coded by genes of the major histocompatibility complex currently focuses on the genes themselves. Progress does continue to be made on the sequencing of the proteins themselves, a very arduous task. Studies on rejected grafts have revealed that organ and tissue-specific antigens do elicit an immune response in the recipient and may, therefore, be targets of the rejection process. As in the case of the HLA antigens, the critical first step in the investigations of these non-HLA antigens is the development of suitable specific reagents. The advent of the cell cloning and of the hybridoma technologies has made possible the systematic preparation of such reagents. Indeed major efforts are currently underway, principally involving monocyte/endothelial antigens, but also involving epidermal antigens.

IMMUNOBIOLOGY AND IMMUNOCHEMISTRY BRANCH

I. Administrative Summary

A. Scope

This Branch is concerned with the biology and chemistry of the immune system and its products. The fundamental studies which it supports on the structure and function of the immune system are directed toward acquiring a complete understanding of immune response mechanisms at their basic cellular and molecular levels as they function in health and disease.

Activities in this broadly based program area cross traditional disciplinary lines of biology and chemistry and encompass anatomic, physiologic, pharmacologic, and microbial biology and organic, physical, and biological chemistry. Its scope includes studies of the origin, maturation, localization, and function of immunologically active cell populations, and the mechanisms involved in the induction, modulation, regulation, and expression of immune reactivity, as well as physicochemical studies of antigens and their homologous antibodies and the mechanisms and kinetics of antigen-antibody reactions.

B. Organization

Research activities supported within this Branch are grouped in the general programs for Immunobiology and Immunochemistry and in a Program of Institute Emphasis (PIE) for Lymphocyte Biology. This Branch also supports a contract activity which provides research reagents and materials to facilitate ongoing research.

1. Immunobiology

This program is concerned with the processes leading to immunocyte differentiation, proliferation, and production of biologically active substances which mediate immune reactions. Research supported within this program includes studies on the origin, maturation, and localization of immunologically active cell populations, the interactions of lymphocyte subpopulations and their interrelationships with macrophages and other leukocytes, the cellular phenomena of antigen processing, immunologic tolerance and enhancement, and the mechanisms involved in the induction, modulation, and regulation of immune responses. The cellular mechanisms involved in the induction, maintenance, expression and pathophysiology of delayed type hypersensitive immune reactivity are also included in this category. Also relevant are studies of the lymphokines and other lymphocyte substances which activate macrophages and have pharmacologic effects on lymphocyte and other leukocytic cell functions such as chemotaxis, phagocytosis, blastogenesis, cytotoxicity, and microbial resistance.

2. Immunochemistry

Research grouped in this program category focuses on the defined molecular components of the immune system with particular emphasis on elucidating the molecular basis of the regulation of the immune response utilizing chemical and physicochemical techniques for study of immunologic reactants and reactions. A major aim of studies supported by this program is to elucidate the structure of antibodies with emphasis on their antigen binding, specificity, and other biological functions. A second objective of this program is to elucidate the critical aspects of antigen structure that determine immunogenicity of the antigen.

3. Program of Institute Emphasis for Lymphocyte Biology

This Program currently supports five program projects, each of which is a multidisciplinary research effort integrating the technical expertise of cell biologists, geneticists, cellular immunologists and immunochemists and is directed by an acknowledged leader in the field. Their combined studies are designed to expand knowledge of the morphologic and functional heterogeneity of lymphocyte populations and to develop the capability for identification and selection of lymphocyte subpopulations with specific immune reactivity or antigenic composition, for hybridization of such populations and for selective production of specific lymphocyte products. The objective of this Program is to acquire as complete a knowledge as possible of the life history of immunocompetent cells and of the physiologic and external factors that determine their fate and function in vivo and in vitro.

This Program of Institute Emphasis represents a major financial and philosophic commitment by the IAIDP and the NIAID to definition of the biology of the lymphocyte and the immune system in general. Support for carefully organized efforts by closely interacting groups, led by well established and highly productive senior scientists, through the program project mechanism has proven to be successful and effective, both scientifically and administratively. This Program was initiated approximately ten years ago with the award of four program project grants, each directed toward the goal of understanding the biology of the lymphocyte and having different but complementary approaches. Since then, three of the original projects have successfully undergone competitive peer review and received renewal of support for a five year period. In addition, two new program projects were established, one each in FY 1978 and FY 1979.

The productivity of these select groups has been exceptional and their varied contributions have been, and continue to be, the stimulus for the intense current efforts in this field. In fact, many of the initiatives identified for concentrated development during this decade in the recent report of the NIAID sponsored Immunology Study Group have been, and are, within the scope of the Lymphocyte Biology Program. In this context, during FY 1981 the Institute considered the future of this Program and decided, with the concurrence of the National Advisory Allergy and Infectious Diseases Council, to continue it in its current form. Accordingly, a Request for Applications was published in March, 1981 announcing a competition for awards in FY 1982. Applications were received from the two active program projects scheduled for competitive renewal and from an additional investigative group. These applications were reviewed for scientific merit in October, 1981 and by the Advisory Council in January, 1982; as a result, renewal of support for the two program projects was awarded in May, 1982. An identical Request for Applications was published in November, 1981 announcing a competition for awards in FY 1983. Applications have been received from the two active program projects scheduled for competitive renewal and from four other investigative groups. These applications were reviewed for scientific merit in June, 1982 and are scheduled for review by the Advisory Council at its September, 1982 meeting.

4. Reagents and Resources Contracts

The contract mechanism has been employed to assist current research efforts by providing useful and unique reagents and materials to investigators.

On recognition of the important functional roles that lymphocyte cell surface molecules exert on the immune system, contract awards have been made for the acquisition and distribution of antisera with specificities directed against various mouse cell surface antigens to facilitate research in this area. A supply of antisera against many of the H-2 gene products of the murine major histocompatibility complex (MHC) has been made available for distribution and antisera specific for antigens of the I region of the H-2 complex have been prepared and are available for distribution. Similarly, the acquisition and distribution of Ly antisera and the preparation of antisera against newly recognized Ly antigens and other immunologically relevant cell surface molecules are being supported by a contract mechanism. Interest in these reagents remains high; approximately 1,000 ml of these antisera were distributed in 235 shipments to 169 investigators during the current year. Of these recipients, approximately 56% were supported by NIH grants; 38 of these investigators were NIAID grantees.

The contract awarded for the acquisition, characterization, storage, and distribution of hybridoma cell lines also is intended to support and facilitate investigator initiated research studies of the immune system. During the first two years of operation, 96 hybridoma cell lines have been acquired; 42 are now available for distribution and 54 are being characterized and propagated for subsequent storage and distribution. The specificities of the antibody products of these cell lines cover a broad range of research interests including among others, antimicrobial, anti H-2, anti-Ly, and anti human lymphocyte cell surface antigens. It is estimated that during the current year, approximately 400 cell lines will be distributed to approximately 110 investigators. Of these recipients, approximately 41% were supported by NIH grants; 33 of these investigators were NIAID grantees.

Support is being provided for continuation of a trans-NIH contract project which serves as a single reference resource and data base for amino acid sequences of immunoglobulins, H-2, Ia, and related human and murine MHC products. Two books containing detailed sequence data, as well as approximately 25 journal publications, have resulted over the seven years during which this project has been active. Current efforts include the continued collection, tabulation, and analysis of amino acid sequences as well as expansion to the nucleic acid sequences of the genes controlling the synthesis of these molecules.

C. Awards and Support Levels

Relevant activities in immunobiology and immunochemistry are supported through various mechanisms including contracts, individual post-doctoral fellowships, institutional pre- and post-doctoral training grants, career and career development awards, as well as investigator initiated research grants.

The following shows the distribution of support by award mechanism for the activities of the Branch during FY 1982.

Immunobiology and Immunochemistry Branch
FY 1982 Awards

Award Mechanism	Number	Amount*
Research Grants	265	$ 30,500,963
Career and Career Development Awards	19	$ 712,555
Subtotal	284	$ 31,213,518
Fellowship Awards	29	$ 504,986
Training Awards	8	$ 850,840
Subtotal	37	$ 1,355,826
Contracts	4	$ 513,813
Total	325	$ 33,083,157

* Total costs, indirect costs estimated

The distribution of these awards by discipline is approximately 2/3 for immunobiology and 1/3 for immunochemistry. Approximately 22% of these awards were for competing new or renewal applications; the remainder represents commitments to support awards made in prior years.

Support for the Lymphocyte Biology PIE is included in the research grant category. During FY 1982, five program project awards, at a total cost of $3,566,749, were made to support this activity.

II. SCIENTIFIC SUMMARY

Fundamental studies of the immune systems are in a transitional phase, shifting from descriptive to analytical approaches, as a result of the application of recently developed technology. The experimental immunologist now has the capability to visualize and isolate specific cellular components of the immune system for precise studies of the molecular mechanisms responsible for their functional activity. With appropriate monoclonal antibodies prepared by hybridoma technology, these cells can be identified and sampled using the fluorescence activated cell sorter. By suitable culture techniques, the selected cells can be cloned and propagated in large volumes to provide a sufficient supply of sub-cellular components for molecular characterization. Isolated fractions can be further defined by analytical techniques, providing detailed structural information and permitting definitions of the nucleotide sequence controlling the synthesis of important cellular constituents. Using this information, relevant molecules can be synthesized in sufficient quantity and purity by recombinant DNA technology to permit detailed studies of their functional role in the immune system. With such capabilities, contemporary immunologists are exploring the molecular biology of the immune system with intensity to acquire a complete understanding of its normal and abnormal function.

A full understanding of the ontogeny of immunocompetent cells is required for selective manipulation of the immune system and for correcting imbalances and deficiencies which compromise host immune defenses. For this reason definition of the origin, differentiative pathways, and functional roles of the various subpopulations of lymphocytes continues to be a focus of considerable investigative effort. Although it is certain that one population of mature lymphocytes (T) is thymus dependent and another population (B) is thymus independent, their progenitors and their mechanisms of functional maturation are still not precisely identified. Studies of T and B cell lineage have been greatly facilitated by the recognition that certain lymphocyte surface molecules are characteristically expressed at specific stages of differentiation and maturation and by the availability of specific antisera to these marker antigens.

Emphasis currently is being placed on B cell ontogeny because it has been recognized that events controlling the expression of immunoglobulin genes occur very early in B cell development, providing the opportunity to identify the genetic mechanisms responsible for immunoglobulin synthesis and diversity, and that many of the clinically relevant abnormalities in antibody producing cells reflect defects which occur and become evident very early in B cell differentiation. Furthermore, monoclonal antibodies against phenotypic and functional markers that define a series of sequential steps in B cell differentiation have been developed and provide the means for separating and studying independently the characteristics and functions of cells at each developmental stage. Transformed cell lines representing many of the stages of B cell differentiation also are available and hybridoma technology has provided the opportunity to arrest and maintain normal B cells at varying developmental stages. It is likely that techniques for developing and maintaining clones of genetically unaltered B cells soon will be available.

Using such approaches, considerable information concerning the regulation of B cell differentiation has been obtained recently. It is now generally accepted that the developmental process leading from multipotential stem cells to pre-B cells to immature B lymphocytes bearing cell surface immunoglobulin (IgM) are genetically programmed and are not antigenically driven. Nevertheless, the respective roles of self or exogeneous antigens, mitogens, or T cell factors, in contrast to intrinsic genetic programs, in mediating succeeding steps in differentiation, particularly the expression of diverse immunoglobulin isotypes, are less well defined.

Analysis of the expression of isotype diversity has contributed importantly to developing current understanding of the process of B cell differentiation. Sequential changes in expression of isotypes in the cytoplasm or on the surface of B cells during ontogeny or after antigenic or mitogenic stimulation have served as the major phenotypic markers for discerning sequential steps in differentiation. In the recent past, the segment of mouse chromosome 12 which contains the family of genes encoding immunoglobulin heavy chains has been analyzed in considerable depth and detail. The resulting information concerning the organization and structure of immunoglobulin genes in their germline configuration and the changes in context and content of these genes that accompany B cell differentiation, coupled with the biological understanding of the differentiation process, has provided the opportunity for gaining a precise understanding of the molecular mechanisms which mediate the process. The experimental data indicate that development of isotype diversity is an intraclonal process that begins with expression of IgM by an immature B cell and leads to a family of plasma cells and memory B cells which express the same immunoglobulin light chains in conjunction with each of the different heavy chain isotypes. Current genetic evidence suggests that isotype switching is accomplished by chromosomal translocation of immunoglobulin gene complexes during differentiation. Further definition of the genetic mechanisms involved in immunoglobulin synthesis and isotype switching may soon provide experimental and clinical immunologists the capability to genetically engineer the system as necessary to either enhance or abolish a specific immunoglobulin isotype.

Although the functional distinction of lymphocyte populations is convincingly documented, the purpose and mechanisms of their interactions with each other and with macrophages is still being examined. It is clear that B lymphocytes differentiate to become antibody-secreting plasma cells and that differentiated T Lymphocyte subsets can exert helper or suppressor effects on B Lymphocytes as well as effector functions in transplantation reactions and in cell-mediated immune reactions to various microbial agents. The cooperative and effector functions of T lymphocytes have been found to correlate well with the presence of the cell surface Ly antigen. It also is known that these functions are controlled and regulated by other cell surface antigens which are products of the genes in the MHC. The regulatory role of MHC products and the functional role of other cell surface antigens in the immune response have been defined most extensively in the mouse although systems analogous to the H-2 component of the murine MHC have been identified in other animals and in man.

Evidence obtained in several experimental systems has convincingly demonstrated that efficient physiological interactions among macrophages, T cells, and B cells require that these cells share membrane molecules encoded for by the MHC of the species. The genes controlling interactions between T and B lymphocytes are located in the I region of the mouse H-2 complex; T Lymphocytes will not exert effective helper functions for B cells when these cells differ at the relevant I region locus. Genetic restrictions also are imposed by products of I region on interactions between macrophages and immune T cells. Naive lymphocytes will respond to an antigen even if it is presented on a histoincompatible macrophage. However, the secondary immune response is genetically restricted; the immune T cell must be stimulated with antigen-bearing macrophages which are genetically identical to the macrophage that presented the antigen during the primary immunization.

The molecular basis for genetic restriction of the immune system is the subject of intense interest now that the technology of gene cloning has been applied to the MHC. The complete nucleotide sequence of several MHC genes has been determined and, within the immediate future, it is expected that the complete molecular structure of representatives of the major classes of MHC genes and the protein products encoded by these genes will be available. The structural definition of MHC gene products will provide the opportunity to clarify the molecular basis of the genetic restriction of immune responses, as well as of the cell-cell recognition process which can play a key role in transplantation, autoimmunity and in resistance to a variety of infectious and neoplastic diseases.

Future applications of gene cloning technology may also clarify understanding of the immunoregulatory functions of T lymphocytes. Functional subsets of T lymphocytes have been identified on the basis their surface Ly antigens. In the mouse, helper T cells have been shown to be Ly1+ while suppressor cells are Ly2,3+; human homologs, both structural and functional, of the murine Ly antigens also have been described. These surface molecules are products of differentiation genes which are outside of the MHC but are believed to be located on the same chromosome.

The eventual cloning of these genes and the structural definition of their products will permit a clarification of their immunoregulatory mechanisms at a molecular level.

Interest also is currently focused on soluble immunoregulatory products of T lymphocytes. It is clear that soluble helper and suppressor factors are released by their respective cells and that these factors are functional when added to antigen-stimulated cell cultures. The potential use of such soluble immunoregulatory factors in the intact host has served as a stimulus for study of the molecular nature and mechanisms of action of these factors. The possibility of achieving immunoregulation with cellular products is exciting because it avoids the recognized complications of cellular transfusions. For this reason, among others, efforts to produce soluble immunoregulatory factors in large volumes and in relative pure preparations are increasing. Hybridoma technology is one possible approach which has met with some success in the murine system; a T suppressor hybridoma has been developed and its suppressor factor has been reported to possess functional and chemical properties identical to those of the parent suppressor T cell.

Another active area in immunoregulation concerns the unique antigenic determinants or idiotypes associated with the variable regions of antibodies, receptors, and antigen-binding factors. These idiotypic markers constitute a target for the regulatory action of idiotype-specific antibodies, T lymphocytes and other soluble factors. Significant progress has been made recently in elucidating aspects of the regulatory process which involves idiotype-antiidotype interactions. Treatment of mice with antiidiotype antibody has been demonstrated to abolish delayed-type hypersensitive reactivity by the action of suppressor T cells; certain antibody responses also have been found to be inhibited by treating mice with antiidiotype antibody. The unique specificity of antiidiotype antibodies adds to their potential for use practically as immunoregulatory agents. Hybridoma technology is now being employed to prepare monoclonal antiidiotype antibodies in quantity for subsequent investigation of their immunoregulatory potential.

The related area concerning interleukins also is receiving considerable investigative attention. The interleukins are products of stimulated cells; lymphokines, also known as interleukin II, are produced by activated lymphocytes while monokines, interleukin I, are released by activated monocytes. These cell products exert multiple biological effects and regulate immunological and inflammatory host responses by serving as intercellular messengers which modulate cellular functions. Their biological activities are immunologically nonspecific and genetically unrestricted but have proven to be useful to experimental immunologists. Interleukin I has been shown to enhance B lymphocyte antibody production, promote lymphokine production and augment thymocyte proliferation. Interleukin II has been demonstrated to have a variety of biologic activities including, among others, non-specific helper function, T cell growth promoting ability, and thymocyte maturation capability. The interleukins represent a valuable tool for study of inflammation and, as individual molecules are isolated from this heterogeneous group, efforts to prepare monoclonal antibodies against them for use as anti-inflammatory agents are anticipated.

The most extensive study of the interleukins is centered on the T cell growth promoting activity of interleukin II. In contrast to B cells, T cells have proven to be difficult to maintain in culture as cell lines or clones. The use of interleukin II as T cell growth promoter has permitted a rapidly developing technology in this area. Cultures of cloned T cells are being developed for large scale production of T cells and products for investigative use. The availability of sufficient quantities of cultured T cell clones should soon permit the isolation of sub-cellular components for biological and biochemical characterization. The elucidation of molecular mechanisms involved in recognition, restriction, and immunoregulation should be accelerated through the availability of cloned T cell lines.

The realization of the results of these fundamental studies is, for the most part, still to come. However, the few practical applications that have developed amply justify the effort and support provided to these investigations. Hybridoma technology, for example, already has supplied a variety of clinically useful reagents. The OKT series of monoclonal antibodies can effectively distinguish human peripheral blood lymphocytes on a functional or differentiated basis. Human helper and suppressor T lymphocytes can be distinquished with these antibodies and they have been employed in the clinical diagnosis of various immunologic disorders. Acute lymphoblastic leukemias have been characterized on the basis of differential expression of OKT-defined antigens and abnormal T cell levels have been detected in patients with multiple sclerosis and systemic lupus erythematosus. Promising results with the use of monoclonal antibodies as diagnostic and therapeutic agents in viral infections also are appearing. Monoclonal antibodies against influenza and rabies viruses have been found to detect serologic differences between isolates which were indistinguishable by previously available diagnostic antisera. Monoclonal anti-rabies antibodies have been demonstrated to be dramatically effective in protecting mice from lethal infections. It is reasonable to expect that, in the near future, applications of hybridoma technology for clinical diagnosis will be extensive and will offer extremely useful approaches to therapy of a variety of immunologic, infectious, and neoplastic diseases.

Microbiology and Infectious Diseases Program

Table of Contents

Report of the Director....................................6-1

  A. Administrative Summary..............................6-2

  B. Scientific Summary..................................6-6

  C. International Health................................6-9

Bacteriology and Virology Branch..........................7-1

  A. Bacteriology..7-2

    Sexually Transmitted Diseases........................7-2

    Hospital Associated Infections.......................7-3

    Streptococcal Diseases and Sequelae..................7-5

    Leprosy..7-6

    Tuberculosis...7-7

  B. Mycology..7-8

  C. Virology..7-9

Clinical and Epidemiological Studies Branch...............8-1

  A. Enteric Diseases Program............................8-1

  B. Closed Volunteer Facility...........................8-3

  C. Collaborative Clinical Trials of Antibiotic Therapy..8-4

  D. Nutrition, Infection and Immunity...................8-6

  E. Rapid Viral Diagnosis...............................8-6

  F. Epidemiology and Biometry Section...................8-7

  G. Infectious Diseases.................................8-7

  H. Immunologic Diseases................................8-9

  I. Biometric and Consultation Research.................8-9

  J. Other Activities....................................8-9

Development and Applications Branch....................9-1

A. Introduction....................9-1

B. Program Summary....................9-2

C. Influenza Program....................9-4

D. Viral Respiratory Diseases Program....................9-5

E. Viral Hepatitis Program....................9-6

F. Antiviral Substances Program....................9-7

G. Bacterial Vaccines Program....................9-9

Pneumococcal Diseases....................9-9

Meningitis....................9-10

Rocky Mountain Spotted Fever....................9-10

Pertussis....................9-11

H. Viral Vaccine Program....................9-11

Molecular Microbiology and Parasitology Branch.............. 10-1

A. Introduction and Staff Activities....................10-1

B. Molecular Microbiology....................10-2

Mechanisms of Resistance to Antimicrobial Agents....10-2

Recombinant DNA Molecular Research....................10-4

Other Basic Research....................10-5

C. Parisitology....................10-6

Biological Regulation of Vectors....................10-6

Immunology of Parasitic Infections....................10-7

General Parasitology Research Highlights..........10-10

Contract Activity....................10-10

Microbiology and Infectious Diseases Program

Annual Report, 1981-82

Director's Report

Administrative Summary

Function and Organization: The Microbiology and Infectious Diseases Program (MIDP) supports research with the broad aim of improving health by controlling diseases caused by infectious or parasitic agents. During FY 1981-82, MIDP operated with the following organizational structure and staff.

Office of the Director (OD)

Director-William S. Jordan, Jr., M.D.
Special Assistant to the Director for International Health-Earl S. Beck, Ph.D.

Bacteriology and Virology Branch (BVB)

Chief-Milton Puziss, Ph.D.
Virology Program Officer-William P. Allen, Ph.D.
Mycobacteriology and Mycology Program Officer-Darrel Gwinn, Ph.D.

Clinical and Epidemiological Studies Branch (CESB)

Chief-Robert Edelman, M.D.
Head, E&B Section-Richard A. Kaslow, M.D., M.P.H.
Biostatistician-William C. Blackwelder, Ph.D.
Clinical Trials-Richard E. Horton, M.D., M.P.H.
Executive Secretary, NIAID-CRS; IND Coordinator and Rapid Viral Diagnosis Program Officer-Martha J. Mattheis, M.A.
Epidemiology Associate-Alfred J. Saah, M.D., M.P.H.
Epidemiology Associate (new appointment beginning July 1 for an academic year at Johns Hopkins School of Hygiene)-Anne Bailowitz, M.D.
Epidemiologist (assigned to NIAID/AID Regional Project on Epidemiol. and Control of Vector Borne Diseases in the Near East)-Fred Feinsod, M.D., Scd.
Epidemiologist/Statistician (Part-time)-Patricia Koslowe, Ph.D.
Statistician-Marie Chang, B.A.

Development and Applications Branch (DAB)

Chief-George J. Galasso, Ph.D.
Antiviral Substances Program Officer-Maureen Myers, Ph.D.
Bacterial Vaccines Program Officer-James C. Hill, Ph.D.
Hepatitis and Respiratory Viruses Program Officer-Franklin J. Tyeryar, Ph.D.
Influenza Program Officer-John LaMontagne, Ph.D.

Molecular Microbiology and Parasitology Branch (MMPB)

Chief-Irving Delappe, Ph.D.
Parasitology and Medical Entomology Program Officer-Harley Sheffield, Ph.D.

Special Recognition: 1) Throughout the year, Dr. George Galasso participated as a candidate in the HHS SES Candidate Development Program, undertaking demanding assignments while continuing to provide leadership for his Branch. 2) Dr. Galasso has served as a consultant to the World Health Organization on interferon, and DAB was recently designated as the WHO Collaborating Center for Interferon Reference and Research with Dr. Galasso as Center Director. 3) Drs. Hill, LaMontagne, Puziss and Tyeryar were elected to membership in the Infectious Diseases Society of America.

Estimated Expenditures:

Awards	Number	Amount
Research Project Grants	909	$ 92,073,000
Research Career Program Awards	44	1,708,000
Other Research Grants	15	527,000
Subtotal, Research Grants	968	94,353,000
Research Training		
Individual Awards	57	984,000
Institutional Awards	36	3,052,000
Subtotal, Training	93	4,036,000
Research and Development Contracts	47	8,633,000
Subtotal, Research and Training		$107,022,000
Other Objects (U.S.Japan travel, program travel, publications, workshops, etc.)		466,000
Total		107,488,000

Workshops: The following workshops were organized by MIDP staff:

October 14	The Role of Prophylactic Penicillin in Control of Early Onset Group B Streptococcal Sepsis in the Newborn. Publication: to be submitted to JID
March 9	Workshop on Interferon Nomenclature Publication: Interferon Scientific Memoranda
August 30-31	Workshop on *H. influenzae* type b Vaccine Publication: summary to be submitted to JID
September 20-21	NIAID Workshop on Enteropathogenic *E. coli* (EPEC) Publication: summary to be submitted to JID
September 22	Reye's Syndrome Workshop Publication: undecided
September 29-30	Workshop on Respiratory Viruses Publication: summary to be submitted to JID

Commercialization of Biomedical Research: Last year's report noted that two technologies spawned in large part by NIAID-supported investigators - recombinant DNA technology for genetic engineering and hybridoma technology for the production of monoclonal antibodies - had presented universities with both the opportunities and risks of new commercial possibilities as scientists and administrators explored mechanisms for collaboration between academic institutions and industrial corporations. During the year, several mechanisms were devised to foster application of these two technologies while at the same time seeking to prevent the profit motive from distorting scientific research and traditional faculty-departmental-university relationships. The implications of the emerging "Academic-Industrial Complex" merit additional comment this year.

University presidents and faculty committees concerned with the development of guidelines to govern agreements between universities and biotechnology companies begin with the perception that the motivations and goals of industry in supporting research in academic institutions are different from those of the federal government as the profit motive is dominant. Approximately $1 billion were spent in 1980 in the field of genetic engineering, a level of expenditure expected to increase fivefold by 1985. Thus, a concern in evolving academic-industrial relations is that research goals may be misdirected by a vision of substantial financial reward. To prevent such distortion, Harvard President Derek Bok has proposed a number of guidelines, including prohibiting a professor from simultaneously holding stock in a company which supports him or being a consultant to it. The Massachusetts Institute of Technology, and Stanford University, among others, have now negotiated agreements involving millions in industrial support, these agreements addressing such questions as patents, licenses, and royalties, the right to publish, and faculty appointments and duties. Among the most notable agreements is the $70 million one between the Massachusetts General Hospital and Hoechst AG which provides support for a new Department of Molecular Biology for ten years; and a $23.5 million five-year research agreement between Washington University and the Monsanto Company. Some agreements have been between scientist and industry; others have been institution to institution. Federal agencies like the NIH will follow these agreements with interest, for they are aware of the fact that absolute and relative decreases in federal funding are making it necessary for academic institutions and their faculties to seek other sources of support for their research and for the training of younger scientists. It is to be hoped that the universities can manage this change so as to maintain the quality of their research and teaching programs while enhancing the effectiveness of technology transfer.

Accelerated Vaccine Development: The first step toward implementation of the Institute's initiative for the Accelerated Development of New Vaccines was taken early in the year when the professional staff of MIDP met for a three-day review of the status of NIAID's vaccine and development program, and reached a consensus as to those vaccines that should be assigned priority for accelerated development. This internal review anticipated external review and evaluation by a group of consultants. Proposals for the latter review were evaluated during the year, and negotiations with the Institute of Medicine are in the final stages.

Following review and discussion of over 30 agents or groups of agents, excluding influenza, the staff updated three developmental listings: 1) development completed; ready for expanded clinical trials; 2)encouraging progress made; further development needed; and 3) early development; basic studies in progress. Concurrently, the agents were placed in three categories for phased, sequential study by consultants: 1) diseases for which safe, effective vaccines do not now exist, but that result in high morbidity, mortality or socio-economic costs in the U.S. population in general; 2) diseases of importance to special subsets of the U.S. population; and 3) diseases of importance to less technologically advanced nations.

The diseases were next ranked according to priority of need in the U.S. and developing countries, and then ranked according to technical feasibility and the prospects for accelerated development using new and emerging technology. A consensus was reached as to how these rankings should be integrated. On this basis, MIDP staff assigned priority to ten agents or agent pairs, five for use in the U.S. and five for use in developing countries, as follows:

For U.S.	International
1. *Haemophilus influenzae*	1. Malaria
2. Gonococcal	2. Typhoid / *E. coli*
3. Parainfluenza/RSV	3. Leprosy
4. Pertussis (improved)	4. Streptococcal, group A
5. Rotavirus	5. Shigella

Progress has been made in each of these areas, as detailed in reports from the four MIDP Branches. Of particular note are the following:

Haemophilus influenzae: Several approaches are being taken to make PRP polysaccharide antigens immunogenic in young children. Discussions have been held with two manufacturers working on this problem, and a third is known to be doing so. A Lederle vaccine composed of PRP plus a small amount of whole cell pertussis vaccine induced protective levels of antibody in 70% of children two to three months of age. Other "adjuvants," e.g., diphtheria toxoid, may be safer and more effective. A workshop on August 30-31, 1981 reviewed the current status of such studies.

Gonococcal: The U.S. Army is seeking final approval for a field trial of a gonococcal pilus vaccine. NIAID-supported research of pilus and outer membrane protein antigens is in progress.

Parainfluenza: A contract has been funded to explore the use of the parainfluenza virus type 3 fusion protein as a protective antigen. A workshop on September 29-30, 1981 reviewed this and other current studies on parainfluenza and RSV vaccines.

Pertussis: The need for an improved pertussis vaccine long has been recognized. Although effective in preventing whooping cough, the current whole-cell vaccine is associated with a greater incidence of undesirable side-effects than other pediatric vaccines. The sixth and final session of an International Symposium on Pertussis in November 1978 addressed prospects for improved vaccines, and included a presentation by Nahase and Doi on a partially purified vaccine prepared at the Japanese National Institute of Health. At a meeting on bacterial vaccines in September 1980, Sato and associates from that institute joined investigators from the U.S. Bureau of Biologics to discuss the role of the two major protective antigens - filamentous hemagglutinin (FHA) and leukocytosis-promoting-factor (LPF-HA) - in immunity to pertussis. Studies then continued in both Japan and the U.S. to further purify and characterize these antigens. Progress toward this end was again reviewed at a BoB workshop in February 1982, sponsored jointly by CDC and NIAID.

In April 1982, a Washington, D.C. television station aired a program questioning the benefits and distorting the risks of pertussis vaccine. In response, the continued routine use of pertussis vaccine was endorsed by the PHS and ACIP, the American Academy of Pediatrics, and the National Advisory Council of NIAID, with the latter emphasizing the need for continued research to develop an equally safe but less toxic vaccine. The new Japanese acellular vaccine, now in routine use in that country, may be such a vaccine; plans are being made to test this "precipitated-purified" pertussis vaccine in the U.S. while U.S. laboratories seek an even more purified sub-unit vaccine.

Rotavirus: Reference should be made to the signficant progress reported by the intramural scientists in the Laboratory of Infectious Diseases.

Malaria: Antibodies specific for *Plasmodium falciparum* sporozoites are detected in more than 90% of West African adults, whereas most samples from children have low or negative reactions. A sporozoite vaccine would ideally induce immunity in children, eliminating the long period required for the development of antisporozoite antibodies under natural conditions. Such a vaccine now seems feasible since monoclonal antibodies can be utilized to purify and characterize sporozoite antigens. One such antigen of *Plasmodium berghei*, Pb44, is a differentiation antigen involved in a unique, presumably essential, function associated with mature sporozoites. Incubation of sporozoites *in vitro* with antibodies to Pb44 abolished their infectivity; antibodies to *P. berghei* sporozoite surface antigen block the entry of sporozoites into cultured cells and confer complete passive protection on recipient mice. An irradiated sporozoite vaccine has induced active immunity in mice. The challenge now is to apply new technology to the development of a *P. falciparum* vaccine for man, i.e., purification of the sporozoite antigen, sequencing and synthesis of the essential peptides, and administration with a safe and effective adjuvant. No mean task! Experimental vaccines also are being developed using other extracellular stages of the parasite-merozoites and gametes.

Typhoid: An attenuated, mutant strain of *Salmonella typhi*, Ty21a, developed by the Swiss Serum and Vaccine Institute, is now licensed in Switzerland. A field trial of this live oral vaccine in Egyptian children showed it to afford a 95% protection rate after three years of observation. Dr. Myron Levine, Director of the NIAID-funded Center for Vaccine Development at the University of Maryland, has simplified oral administration, and is conducting a field trial in Chile. A safe, effective typhoid vaccine is needed for members of the U.S. Armed Forces, for American tourists, and for the developing countries.

Leprosy: Two glycolipid antigens specific to *Mycobacterium leprae* have been characterized as triglycosyl phenolic phthicerol diesters with trisaccharide chains specific to *M. leprae*. Glycolipid I, found in abundance in the bacillus and in infected tissue, reacts specifically with sera from leprosy patients. Its use as a skin test antigen should facilitate epidemiological studies, including the identification of susceptibles. It is likely to be an essential component of any vaccine.

II. Scientific Summary

Progress reported by extramural scientists is summarized in the reports which follow, and will not be redistilled here. Rather, it is timely to reflect on the changing patterns of infectious diseases in the U.S., flowing from both past medical progress and dramatic changes in human behavior and social patterns. Remarkable progress in the prevention and treatment of infections has been made in this century as a result of man's ability deliberately to alter his internal (biologics, drugs, nutrients) and external (housing, pesticides, sanitation) environments. The control of the infectious diseases of childhood has been a major factor (in the U.S.) in assuring an average life expectancy beyond the proverbial three score years and ten. But grand-parents don't babysit anymore; mothers work; young children are placed in daycare centers. Increasing numbers of elderly persons are hospitalized to have diseased parts removed and worn out parts repaired or replaced. Both settings foster the spread of infectious agents, for man is still surrounded by a myriad of microbes. Only smallpox has been eradicated. The remaining pathogens, even those with only a few small strands of nucleic acid possess the ability to outwit man by becoming resistant to antibiotics, flourishing on super-absorbent tampons, or growing in the water of cooling towers.

Thanks to penicillin, few patients insane as a consequence of neuro-syphilis are in mental hospitals. Yet infectious syphilis is resurgent, along with all other sexually transmitted diseases, accompanied by an increase in the number of cases of congenital syphilis. Thanks to streptomycin and isoniazid, the estimated prevalence of tuberculosis in the U.S. has been reduced to 24.4 per 100,000, and nearly all tuberculosis hospitals have been closed. Yet refugees have entered the U.S. with prevalence rates of 650 (Haitian) to 926 (Indochinese). A high proportion of the culture-proved cases were excreting organisms resistant to isoniazid. Antibiotic usage in the Philippines and Africa influences the treatment of gonorrhea and pneumonia in the U.S. It is truly one world.

Daycare Centers: A major social change in recent years has been the proliferation of daycare center facilities in the U.S. to care for the young children, often still in diapers, of working mothers. Nearly two million children are enrolled in centers that care for more than 12 children and offer ideal settings for the spread of enteric and respiratory infections. Outbreaks of hepatitis A, meningococcal disease, invasive Haemophilus influenzae type b infections, tuberculosis and diphtheria have been reported. A few NIAID-supported investigators have initiated studies to define the epidemiology and means of control of infectious diseases in daycare centers. Much more needs to be done.

Hospital-Acquired Infections: Of the 40 million people hospitalized in the U.S. each year, approximately 5 to 6 percent - 2 to 2.5 million - develop a nosocomial infection. These infections are directly responsible for 20,000 deaths and indirectly responsible for 60,000 others. Nosocomial infections account for about 6 million extra patient days per year at an additional cost of $1 to $2 billion. Aerobic gram-negative bacilli cause 60% of such infections, and outbreaks caused by multiply-resistant strains have increased in frequency in recent years. There has been an increase in outbreaks caused by methicillin-resistant Staphylococcus aureus, by fungi, particularly candida and aspergillus species, and, most notably, by several species of Legionella. The aging of the population, the greater use of invasive technology, and the increasing use of immunosuppresive therapy can be expected to perpetuate, if not exacerbate, the problems of hospital-acquired infections. It is anticipated that the results of the SENIC Study, soon to be published by CDC, will provide new guidelines for research in their prevention and treatment.

Acquired Immunodeficiency Syndrome (AIDS): A new syndrome that encompasses nearly all of the programmatic responsibilities of NIAID - immunology, bacteriology, mycology, parasitology, virology - was reported with alarming and increasing frequency during the year. A feature that all patients shared was unexplained immunosuppression. All other manifestations were secondary to this, e.g., opportunistic infections, especially *Pneumocystis carinii* pneumonia, disseminated Kaposi's sarcoma, and persistent, generalized lymphadenopathy. Since June 1981, nearly 500 cases have been reported, with over 75% in homosexual men. This association led to designations such as Gay-Related Immunodeficiency Disorder (GRID) and Gay Compromise Syndrome (GCS), promptly reduced to the Gay Plague.

Early suggestions as to etiology included inhalation of amyl nitrite, a drug commonly used to enhance orgasm and relax the anal sphincter, and infection with cytomegalovirus (CMV), almost universal among homosexuals. Both agents are immuno-supressants; perhaps the combination was responsible. But no. The disease has now occurred among heterosexual men and women, drug abusers who take drugs by injection, Haitians who recently entered the U.S., and patients with hemophilia A receiving periodic injections of factor VIII.

In addition to CMV, the microbial species shown to cause disease in these patients have included herpes simplex virus, *Mycobacterium tuberculosis*, *M. avium*-intracellular, *Klebsiella pneumonia*, *Candida albicans*, *Cryptococcus neoformans*, *Toxoplasma gondii*, and *Entamoeba histolytica*. These pathogens, as with pneumocystis, are mostly those that would be expected to be found in patients with defective cellular immunity. Indeed, patients have shown a selective depletion of the T inducer T cell subset with a decrease in the OKT4+/OKT8+ ratio. Of patients with Kaposi's syndrome, 61% carry the gene for the HLA-DR5 haplotype as compared to 18% to 20% of the general population. Accordingly, it has been suggested that the syndrome is more properly designated an epidemiologically restricted disorder of immunoregulation.

Because the epidemiology of AIDS so closely resembles that of hepatitis B, and because clusters of cases have suggested person to person spread - and person to blood to person spread - a vigorous search is now underway for an infectious agent, perhaps a new species of human virus. It is regrettable that within NIH no extra-mural mechanism exists to permit a rapid response to such research opportunities.

Drug Abuse: In addition to hepatitis B, and perhaps the putative agent of the acquired immunodeficiency syndrome, addicts who inject drugs infect themselves with a wide variety of microbes. *Staphylococcus aureus* is the most common infecting organism, with a variety of gram-negative rods next. Both the equipment and drugs are contaminated. In one study, 31% of 49 heroin samples cultured and 56% of paraphernalia cultures were positive. Bacillus species, the most common isolates, were recovered from 13 of 20 positive cultures; 5 of the 13 (38%) were identified as *B. cereus*. Other organisms included *Staphylococcus* and *Escherichia coli*. All isolates were resistant to penicillin and the cephalosporins. The health care problems of this segment of society are staggering. *Genital Herpes*: Each year, 300,000 to one-half million cases of herpes genitalis are added to the 20 million already infected, reflecting an epidemic that has attracted nationwide attention in both print and television media. Acting on the recommendations of the NIAID Virology Task Force and the NIAID STD Study Group, MIDP began planning over two years ago to expand research on the epidemiology and natural history of genital herpes. Last year, two workshops, one on antiviral therapy and one on vaccine development, reemphasized the need for such studies. During the year, when program-controlled funds became available, MIDP was able to invite applications and, eventually, to award one grant to Emory University. This was the only new grant supported program-originated initiative funded in FY 1982.

The antiviral agent acyclovir (Zovirax) was licensed for use in the topical treatment of primary, or initial, herpes genitalis infections. Given systematically, the drug prevents recurrences in immunosuppressed patients, but it does not cure since it does not eradicate the latent state. Fear has been expressed that widespread use may lead to the emergence of an increased number of resistant organisms a possibility being studied by MIDP-supported scientists.

Toxic Shock Syndrome: As reported last year, toxic shock syndrome (TSS) has been attributed to toxin(s) elaborated by *Staphylococcus aureus*, and studies have now indicated that these particular strains were imported in the early 1970's. By April 30, 1982, there had been a total of 1660 reported cases, including 88 deaths In 1981, 85% of TSS cases reported were associated with menstruation and 98% of these women were using tampons. Why tampons, particularly high absorbency ones, promote the growth of toxigenic staphylococci is not known, although it has been suggested that the bacterial enzyme beta-glucosidase breaks down the absorbency material - carboxy-methyl cellulose to glucose, providing substrate for the staphylococci. During the year, the Institute of Medicine, with partial support from NIAID, held a workshop on TSS. Subsequently, an IOM committee recommended that women, especially adolescents, should avoid the use of high absorbency tampons to lessen their risk of developing TSS.

Antibiotic Resistant Gonococci and Pneumococci: Strains of penicillinase (beta-lactamase) producing *Neisseria gonorrhoeae* (PPNG) continue to spread throughout the world. In the U.S., the number of cases of PPNG infection increased from 328 in 1979 to 1099 in 1980, and to 2750 in 1981. During the first five months of 1982, 1691 cases were reported, and the infection was considered to be out of control in Los Angeles, Miami, and New York. In the latter city, cases of PPNG infection were being reported at the rate of 100-150 cases per month. It has been estimated that the cost of treatment will be increased by $8 million if PPNG became dominant in the U.S. Of the plasmids identified from beta-lactamase-producing strains, two, the 4.4 megadalton penicillinase plasmid and the 24.5 Mdalton transfer plasmid originated in Southeast Asia, and one (3.2 Mdaltons) in Africa.

The first multiply-resistant isolates of *Streptococcus pneumoniae* were reported from South Africa. They have now been reported from four other countries. Such strains often exhibit resistance to penicillin, chloramphenicol and tetracycline as well as to other antibiotics (e.g., ampicillin, methicillin, carbenicillin, cephalothin, and erythromycin). So far, the isolates have been clustered in a small number of serotypes, including 6A, 6B, 9L, 14, and 19A. The development of antibiotic resistance by gonococci and pneumococci has intensified efforts to develop a gonococcal vaccine and to improve the recently licensed pneumococcal vaccine.

International Health

International health research programs are administered by Dr. Earl Beck, Special Assistant to the Director, MIDP, in conjunction with individual program officers.

International Collaboration in Infectious Diseases Research (ICIDR) is a program initiated in September, 1979. The program is divided into Part A, International Program Project Grants, and Part B, International Exploratory/Developmental Research Grants. The research emphasis of the ICIDR program is infectious diseases and the immunology of these diseases, and includes malaria, schistosomiasis, filariasis, trypanosomiasis, leishmaniasis, and leprosy.

Part A, International Program Project Grants, embraces broadly based multidisciplinary research programs with a well-defined central focus or objective, and with the major portion of the research (80%) being conducted overseas with an acceptable foreign affiliate in a developing country. There are currently five program projects; four have completed the 03 year, and one has just completed the 02 year.

Part B, International Exploratory/Developmental Research Grants, is designed to encourage the individual investigator to develop a biomedical research program with an overseas affiliate, with the major portion of the work performed in the host country. These are planning type grants that may lead to the development of a large program project (P01) or a regular research grant (R01), or a foreign grant (F). Currently, three of the five grantees are completing their third and final year of the granting period. The other two will terminate after the 04 and 05 grant years.

Although the ICIDR program is still in its infancy, less than three years old, the collaborative effort appears to be firmly established and, in general, functioning according to plan. This statement is supported by staff visits to four of the program project grants and two of the exploratory/developmental grants.

Program Project Grants

Harvard University, AI 16305 - Doctors Beck and Edelman travelled to Salvadore, Brazil to visit the Harvard University project, "Endemic Chagas' Disease in Brazil," with Dr. Thomas Weller as the U.S. Prinicipal Investigator. The Brazilian collaborators for the Harvard project are associated with the Federal University of Bahia and with the Oswaldo Cruz Foundation Laboratory (FIOCRUZ). The field area is located in and around the small town of Castro Alves, about 150 kilometers west of Salvadore, an area with the highest known prevalence rate of Chagas' disease in the world; approximately 60 percent of the residents age 20-55 are seropositive for *T. cruzi* antibody. Some time was spent with Dr. Henoir Rocha, Vice Rector for Research and Post-Graduate Education and the scientific administrator for the Brazilians on the Harvard project. Although Drs. Weller and Rocha had some administrative differences during the earlier part of the grant, these have been resolved, and the project appears to be moving along smoothly. Doctor Rocha reassured us that the program was thoroughly integrated and that he was pleased with the scientific progress.

The Harvard laboratory at the Federal University of Bahia is functional and well-equipped. Doctor Charles Todd, an immunoparasitologist, provides the day to day management of the laboratory. He has established the ELISA test for serological identification of Chagas' disease. This test has simplified and accelerated all studies on Chagas' disease. Dr. Todd and collaborators hope to develop an ELISA test for detecting circulating T. cruzi antigen to circumvent xenodiagnosis, which is cumbersome and slow (weeks instead of hours). Monoclones and their corresponding antibodies developed by Dr. Hoff at Harvard will be used by Dr. Todd to identify strains of T. cruzi collected in different parts of Latin America.

The Professor Edgard Santos Hospital, administered by the University, houses the cardiology unit which is directed by Dr. Armenio Guimarães who is engaged in what promises to become a classical study of Chagas' cardiomyopathy. The field studies are carefully planned and coordinated by Dr. Eduardo Mota, a Brazilian epidemiologist trained at Harvard. Patients in the Castro Alves field area were visited and observed in their mud-stick, thatched roofed homes and in a local hospital. In one house, triatomines, the insect vector of Chagas', were observed. The on-site coordinator, Dr. Joseph Piesman, speaks Portugese fluently and has become thoroughly acquainted with work published in old and new Portugese scientific literature. He is collaborating with Dr. Italo Sherlock at FIOCRUZ, and their entomological studies provide valuable information to control units working in the study area. Dr. Piesman is a very able coordinator and an enthusiastic scientist.

Staff was pleased with the over-all performance of the Harvard-Bahia groups. They have been productive in the past, and it is expected that the present level of performance will be exceeded in the next two years. Approximately, 10-12 papers are in preparation or in press.

Cornell University, AI 16282 - Drs. Thomas Jones and Warren Johnson and staff from Cornell, and collaborators from Universities in Bahia and Brasilia came to NIH to make their scientific presentations, since these investigators would not be available when Drs. Edelman and Beck visited the project in Salvadore. This briefing was of enormous benefit in preparing the visitors scientifically for future discussions with the on-site investigators.

Prior to initiating discussions with the Cornell and University of Bahia investigators, a courtesy call was made to Dr. Henoir Rocha. There was no doubt that Dr. Rocha headed the project in Brazil, and this arrangement appears to be functioning smoothly. He is knowledgeable, enthusiastic, ambitious, and determined to continue the leishmania studies regardless of future funding. He hopes to make the University of Bahia the center for leishmania research in Brazil. The on-site U.S. coordinator, Dr. Steve Reed, is based in the FIOCRUZ laboratory in Brotas, where he coordinates the lab and field studies; he speaks Portugese fluently and interacts very well with the Brazilian investigators and patients. Dr. Reed is largely responsible for building and maintaining the small animal colony at the FIOCRUZ. The major portion of the work at the FIOCRUZ is concerned with the immunology and biochemistry of leishmaniasis.

One afternoon was spent at the Professor Edgard Santos Hospital being briefed by Bahian University investigators working on the genetics and clinical aspects of American Visceral Leishmaniasis (AVL or Kala-Azar). The genetic studies of AVL are carefully designed and the results, once obtained, should be definitive whether or not a genetic risk factor is found to be associated with AVL. The immune studies are concerned with lymphocyte transformation, suppressor cell concentration, T helper and B cell activity.

The field studies in Jacobina on AVL are proceeding smoothly with interested and dedicated investigators. A prospective longitudinal field study of AVL has been in progress several years, and the data already collected provided new insights into the natural history of the disease. Staff was on-site and saw the workup of a two year old boy with AVL. The bone marrow smear was teeming with the parasite, L. dononani.

The next site visited was Três Bracos, a village situated in a lush tropical rain forest valley. The surrounding hills are hyperendemic for L. braziliensis infections which cause cutaneous and mucocutaneous leishmaniasis. Two new patients with large ulcers of the extremities were seen at the local clinic. About 60 per cent of the Três Bracos families have had at least one family member infected.

In summary, staff was quite satisfied with the Cornell project. The investigators of both countries are enthusiastic and pursuing their research with vigor. The two field sites are well-coordinated with laboratory and clinical backup in Salvadore and New York.

Michigan State University, AI 16312 - This is a collaborative project among biomedical researchers at the Michigan State University College of Veterinary Medicine, Central Laboratories of the Khartoum Ministry of Health, and the Faculty of the University of Khartoum.

Doctor Frank Neva, Chief, LPD, visited the Sudan to determine whether it might be feasible to do a collaborative study on leishmaniasis in this country and to attend a one-day review in Khartoum of the Michigan State University ICIDR project in the Sudan. Approximately 30 people were at the review, including quite a few Sudanese with no direct involvement in the project. Presentations regarding administration of the program were made by Drs. Hassan Sherif and Jeffrey Williams, and technical reports on schistosomiasis were given by Drs. Hassan Ali, Kardoman, Abdel Gaafar, and Bennett; on onchocerciasis by Drs. Paul Lino and Williams; and on malaria by Dr. Williams vice Jensen, who could not attend because of teaching responsibilities. It was the opinion of Dr. Neva that the review of the MSU program went very well and was quite a success in the eyes of the locals as it gave them an opportunity to publicly demonstrate their participation and collaboration. Dr. Neva was pleased to see the degree of discussion and argument among the locals on various aspects of the oltipraz drug trials. To date, this project has been very successful in working under very difficult conditions. Dr. Jensen recently published an article on his malaria research in Science. He found that sera collected from individuals living in malarious regions of the Sudan not only contained merozoite-blocking antibodies, but also caused intracellular parasite deterioration and classical crisis forms in cultures of P. falciparum. In addition, there are six papers in press and twelve abstracts of papers presented or to be presented at national meetings.

University of Illinois - AI 16308 - This project concerned with various aspects of leprosy was visited briefly by Dr. Karl Western. Although a formal review of the scientific program was not conducted, he did see the facilities at the Chiang Mai University, the McKean Rehabilitation Institute and visited a resettlement village for leprosy patients and their families. All appeared to be integral parts of the proposed studies. He was of the opinion that the epidemological studies were progressing nicely and that the necessary base data were being collected. Dr. David Scollard arrived as the on-site administrator in August, 1981. Doctor Western knows this man personally and feels that he is well-qualified to direct this collaborative research program. The co-principal investigators report that his managerial and scientific expertise has already increased the productivity of the project. A recent progress report from the grantee provides preliminary but promising data on studies of the immunopathology of the disease.

Tulane University - AI 16315 - This program project grant completed its 02 year in May, 1982, since it was awarded a year later than the other four. Staff has not visited the off-site facility in Cali, since the award of the present grant. However, it was visited as an ICMR, and staff is familiar with the physical facilities which are excellent. The collaborative effort is evident in the excellent and extensive progress report submitted for the 02 year. The investigators appear to have designed a well-balanced program which includes epidemiology, development of animal model systems, parasite propagation in tissue culture, and immunological studies of filariasis and trypanosomiasis. The ELISA test is being used to serologically test patients from endemic areas with W. bancrofti. Strains of T. cruzi and T. rangeli have been cloned and will be re-evaluated biologically and for isoenzyme characters, prior to large scale production of stage specific antigens.

Exploratory/Developmental Grants - The two grantees with their overseas sites in Rio de Janeiro were visited by staff while attending the First International Conference on the Impact of Viral Diseases on the Development of Latin American Countries and the Caribbean Region.

Columbia University - AI 16314 - The Federal University of Rio de Janeiro which is the off-site location for Columbia University was visited by Dr. Beck. The laboratories are well-equipped and busy. Although there are some personality problems between the on-site U.S.and Brazilian coordinators, the investigators have been productive, and there were some suggestions made to resolve the differences. This grant will complete its 03 and final year in March, 1983. Work on surface receptors of Leishmania continues.

University of South Florida - AI 16287 - Dr. Gary Rodrick is collaborating with Dr. Lobato Paraense at the Fundacão Oswaldo Cruz Foundation. Although Dr. Rodrick was not able to be present at the time of the staff visit by Drs. Edelman and Beck, Dr. Paraense showed us his excellent laboratories and enthusiastically described the collaborative studies with Biomphaloria straminae. This snail can be infected with Schistosomia mansoni. Since this snail is prevalent in large numbers in and around the Trans-Amazon highway, which opens large areas of land for settlement, it is important to determine its role in the transmission of schistosomiasis. The collaborative relationship between Drs. Rodrick and Paraense is excellent, and in this instance, it is evident that Dr. Rodrick is obtaining invaluable training from his Brazilian collaborators.

Ohio State University - AI 16285 - This grant is completing the 03 year of a three year award. However, because of an industrial action (a strike) by the faculty, work on the project at the University of Nigeria, Nsukka was discontinued from September, 1981, until January, 1982. Doctor Briggs has requested a 6-months no-cost extension to complete the field work and to write manuscripts on the data obtained on the biological control of vectors of human diseases.

University of Washington - AI 16290 - The grantee and his collaborators in Mexico have targeted their research toward the serodiagnosis of subclinical leprosy. During the past year, the suface proteins of live M. leprae have been labeled with ^{125}I using either lactoperoxidase or the reagent Iodogen; and each method indicates that the organism possesses five major surface proteins with subunit molecular masses of 794,000, 53,000, 44,000, 36,000 and 31,000 daltons. A 36,000 dalton protein was found in M. leprae, M. bovis BCG and M. lepraemurium, and might represent a shared antigen.

The arabinomannan antigen was used in the ELISA immunoassay to test serum from armadillos injected with M. leprae. The assay detected antibody in an infected armadillo 10 months after the initial challenge, but remained negative in an animal that did not develop disease. This is further evidence that this antigen, and possibly others, might be used for detecting subclinical cases of leprosy.

The University of Hawaii - AI 16273 - The grantee has continued his research on the in vitro cultivation of P. falciparum. A continuous culture of P. falciparum has been maintained in Honolulu and in Aligarh, India for over one year and has been designated as the FCH-13 strain. This strain continues to produce gametocytes in continuous culture which has facilitated experimentation on gametocytogenesis. Studies are in progress to develop a continuous culture of P. vivax, the predominant mosquito in and around Aligarh.

The U.S.-Japan Cooperative Medical Science Program - This program has been operative since 1965, and provides the basis for collaborative research between scientists of the two countries on diseases of importance to Asia, although some are common to the United States and Japan as well as worldwide. For each disease or research area, five scientists are appointed in each country to provide expert advice and guidance. With the establishment of an Immunology Board in 1981, the program now has one Board and eight Panels. In addition to immunology, the following program areas are included: cholera, environmental mutagenesis and carcinogenesis, hepatitis, leprosy, malnutrition, parasitic diseases, tuberculosis and viral diseases.

The Joint Committee meeting was held in Tokyo, Japan on July 22 and 23, 1982. At this time the formal review of the Leprosy and Parasitic Diseases Panels was completed and that of the Tuberculosis and Viral Diseases Panels was initiated. It was recommended that the Leprosy and Parasitic Diseases Panels be continued. Additional discussions of the Leprosy, Tuberculosis and Parasitic Diseases Panels were held. Recognizing the common scientific interests of the Leprosy and Tuberculosis Panels, the Committee recommended that beginning next year these two Panels hold combined meetings. Also, it was the consensus of the Committee that the guidelines of the Parasitic Diseases Panels be reviewed, and perhaps, expanded and updated to accommodate new technologies and changing needs. Preparatory to such action, a symposium will be held in conjunction with the Joint Committee Meeting in 1983. Experts will be invited to present current scientific reviews for such parasitic diseases as malaria, leishmaniasis, trypanosomiasis, in addition to schistosomiasis and filariasis. Discussions continued on options for reducing program costs without compromising the quality of science. It was agreed that annual meetings be held alternately between Japan and the United States. When possible, Joint Meetings will be held in association with large domestic or international meetings in the two countries. The number of attendees and format of the meetings will be variable to provide maximum program flexibility. Careful planning in program scheduling should provide avenues for reducing program associates costs.

BACTERIOLOGY AND VIROLOGY BRANCH

The mission of the Bacteriology and Virology Branch is development and support of a wide array of programs in biomedical research; these include program project grants, individual research grants, career development awards, training programs of both institutional and individual postdoctoral fellowships, and contracts for targeted research in the fields of bacteriology, virology, and mycology. The Branch is headed by Dr. Milton Puziss, who also administers the Bacteriology Program. The Virology Program of the Branch is headed by Dr. William P. Allen, who also serves as Executive Secretary of the Panel on Viral Diseases of the U. S.-Japan Cooperative Medical Science Program. The Mycology and Mycobacteriology Programs are administered by Dr. Darrel D. Gwinn, who also serves as Executive Secretary of both the Panel on Leprosy and on Tuberculosis of the U.S.-Japan Cooperative Medical Science Program.

Approximate Level of Support

Bacteriology and Mycology

Activity	Number	Amount
Research Grants	223	$18,330,644
Research Program Projects	6	1,950,901
Research Contracts	6	582,117
Career Awards	8	296,153
Training Grants	17	1,271,851
Fellowships	7	115,259
Total	267	$22,546,925

Virology

Activity	Number	Amount
Research Grants	189	$19,843,917
Research Program Projects	2	741,897
Research Contracts	1	0
Career Awards	13	467,114
Training Grants	9	1,167,723
Fellowships	10	175,078
Total	224	$22,395,729

Branch

	Number	Amount
Total	491	$44,942,654

Program Summary

The program of this Branch focuses upon projects in both fundamental and applied biomedical research, with the ultimate and most significant objective being the translation of the knowledge gained into better and more practical methods for the diagnosis, prevention and therapy of bacterial, fungal and viral diseases. Institute programs of significant interest to the Congress are those on Sexually Transmitted Diseases (including research on genital herpes) and on Hospital

Associated (nosocomial) Infections. The biology and biochemistry of viral infections of humans and animals continue as subjects of considerable research interest. Pathogenic mechanisms of acute viral diseases of man and mechanisms by which viruses can initiate and sustain persistent infections are areas of special concern and attention. Under the auspices of the U.S.-Japan Cooperative Medical Science Program certain disease areas have been singled out for special attention. These include research programs on tuberculosis and leprosy, as well as rabies, dengue and other arthropod transmitted diseases, infections that are still common in the world's developing countries.

Bacteriology

Sexually Transmitted Diseases

The sexually transmitted diseases constitute a major public health problem. No significant lessening of the overall problem is seen, although a slight leveling off of the gonorrhea rate has been noted. This disease continues as one of the major epidemic areas of concern, but it has now been equalled, if not superseded by genital herpes and by chlamydial infections. These latter two problems have shown an alarming increase in the United States; the rate of syphilis infections has also risen in recent years. Definitive data are lacking, however, for all the STD problems. A significant increase in genital herpes is of concern to public health officials because of the potential for serious damage or death to infants born of mothers infected with herpes at the time of delivery. There is also increasing concern regarding other sexually transmitted diseases, such as viral hepatitis, amebic dysentery and shigellosis, that are now being seen with increasing frequency, particularly among homosexual groups.

P01 AI 12192-07 K. K. Holmes (University of Washington): Isolates obtained from outbreaks of penicillinase-producing *Neisseria gonorrhoea* (PPNG) infections in Washington State, Louisiana, and the Far East were studied by auxotyping and serogrouping by the coagglutination method. The data indicated that the Washington and Louisiana outbreaks resulted from the spread of imported PPNG strains rather than by transmission of penicillinase encoding plasmids to indigenous U.S. gonococcal strains. The Louisiana outbreak involved a single strain of *Neisseria gonorrhoea*, and probably originated from a common source. On the other hand, several types were involved in the multiple-source Washington outbreak, indicating repeated introduction of new strains of the gonococcus. In other studies by this group, chlamydial infections in pregnant women were identified as a major risk factor for stillbirth or neonatal death. Thirty three percent of pregnancies of infected women in the study group resulted in stillbirths or neonatal deaths, as compared to 3.4 percent of pregnancy outcome in uninfected women. Duration of pregnancy in infected women was also shorter by about 3-4 weeks. The mechanism by which *Chlamydia trachomatis* could cause prematurity and perinatal death requires further investigation.

AI 14262-05 S. Sell (University of California, San Diego): Dr. Sell's data show that systemic spread of syphilis infections in rabbits occurs primarily in lymphoid organs, causing a prompt immune response of both cellular and humoral systems. Organisms were cleared from the infected sites 10-14 days after infection; this was associated with the evolution of the inflammatory reaction primarily from a T-cell infiltrate. Resolution occurred after 17 days, with later foci of tubular atrophy and fibrosis. Long lasting T-cell memory to *Treponema pallidum* cell sonicates, as well as good serum antibody titers, were found. Conclusions were that a prompt and long lasting immune response to T. pallidum in experimental syphilis infections occurs with little or no evidence for any type of immune suppression. T-cell initiated macrophage-mediated destruction appears to be the main mechanism for destruction of the infecting organism. These data are in contrast to reports by others regarding suppression of the CMI response in syphilis.

AI 16585-03 T. Fitzgerald (University of Minnesota): This project's emphasis is on the pathogenesis of syphilis infection, particularly the effects on neural tissue. Dr. Fitzgerald reports that in rat embryonic nerve cell cultures exposed to T. pallidum, the organisms attached to the nerve cell bodies and the dendritic processes; attachment was mediated through the treponemal tip, with no damage to the cell seen at the attachment site. Organisms incubated with dorsal root ganglion cells in culture showed nerve cell disruption on long incubation; disruption of the cells, with cytoplasmic holes, was followed by coagulation of nuclear material and degeneration of the cytoplasm. These observations may be the basis for the painless nature of the majority of syphilitic clinical manifestations as well as the severe nerve damage of late syphilis. High concentration of treponemes in cell cultures of rabbit skin, testis, brain capillary and cardiac muscle showed cell attachment followed by disruption; this suggests the possible presence of some type of toxin-like activity associated with the treponemes. Non-pathogenic treponemal strains did not attach, and immune serum effectively blocked attachment of T.pallidum to capillary tissue. These findings represent an important advance in study of the pathogenesis of syphilis infections.

P01 AI 16959-02 J. C. Hume (Johns Hopkins University): In a subproject of this STD Research Project that deals with human genital papillomas, five distinct subtypes of human papilloma virus (HPV) genomes were identified in genital warts. The viral DNA was detected in all of the acuminate warts examined even though only 30% contained detectable papilloma virus capsid antigen. Viral DNA was isolated from pre-malignant and malignant genital tract tumors; the possible role of HPV in the etiology of cervical and vulvar carcinomas is being investigated. Other studies revealed that the HPV capsid antigen was detected in 16% of the juvenile onset and 25% of the adult onset laryngeal papillomas studied. The HPV DNA was identified as HPV-6. Approximately 50% of condylomas (genital warts) studied exhibited the HPV antigen. This was present in condylomas of both flat and papillary morphology at all genital sites. The absence of detectable viral antigen in as many as half of characteristic papillomas suggests that virus synthesis, while probably essential for transmission, may not be required for morphogenesis of the papilloma. Attempts to propagate the virus on human cell cultures have so far been unsuccessful.

Hospital Associated Infections

Nosocomial infection has long been recognized as a major cause of morbidity and mortality in immunocompromised patients. These infections result in prolonged hospitalization; excessive health care costs inevitably result from these infections, usually by opportunistic gram negative bacteria. The problem is further compounded by a significant trend towards antibiotic resistance among these microorganisms.

Research Highlights

AI 16108-03 E. J. Ziegler (University of California, San Diego): Dr. Ziegler's research goal is to discover the mechanisms by which Pseudomonas aeruginosa invades and destroys blood vessels in the agranulocytic host. This characteristic lesion of P. aeruginosa infection occurs at the primary site as a result of bacteremic spread. Vascular necrosis is mediated by the bacteria alone since no host inflammatory cells are seen; the affected tissue becomes isolated from antibiotics and host defense mechanisms. Recent data on development and use of non-motile Pseudomonas mutants showed that although motility may play a role in invasiveness, it is not required for development of vasculitis. Passive migration of the causal organism with tissue fluid is not important, nor was vasculitis affected by different levels of tissue oxygen. Invasiveness may require motility and extracellular enzyme production, but vasculitis seems to depend on other, yet unidentified properties of the bacterial cell. These findings parallel clinical data that Pseudomonas bacteremia in neutropenia and burns is associated with high mortality irrespective of bacterial phenotype.

AI 13120-05 C. D. Cox, Jr. (University of Iowa): Dr. Cox is studying the factors that enable P. aeruginosa to grow in mammalian tissues. A key determinant of their growth potential is the ability to obtain iron during infection. In previous reports, three different pigments of the organism stimulated iron transport from the extracellular environment. The Pyochelin pigment, a siderophore, possesses a structure unique among bacterial siderophores. All three of the pigments stimulate growth of P. aeruginosa under iron-limiting conditions. Pyochelin stimulates growth of virulent pseudomonas; the net effect is an increase in lethality of the virulent strain. This is, therefore, a virulence mechanism that stimulates bacterial growth by providing the infecting bacteria with iron from the host. In a separate epidemiological study, the pristine environment of a new surgical unit was colonized with Pseudomonas one week after transfer of patients from the old unit; the serotypes and pyocyanin types were found to be the same in the new and the old units.

AI 17609-01 R. Yolken (Johns Hopkins University): Dr. Yolken is studying methods for rapid enzymatic diagnosis of microbial infections. A newly-developed non-radioactive substrate was used in a novel assay for detecting directly beta-lactamase in body fluids. Detection of 10^{-4} units of the enzyme was possible in the C.S.F. of patients with meningitis and in blood of patients with staphylococcal septicemia. Detection of glycosidases in clinical specimens was accomplished by a fluorimetric method; this is of value in detecting fungal infections in children with cancer or other serious disease. The concentration of circulating alpha-galactosidase correlated with the severity of the fungal infection. This simple, rapid assay has markedly improved effective early diagnosis and treatment of these infections. Additionally, a technic was developed for detecting bacterial polysaccharides in a rapid immuno assay using enzyme-labeled antibodies. Using this method, H. influenzae was detected in infected patients, as well as minute concentrations of pneumococcal antigen in clinical specimens.

K04 AI 00391-03 R. J. Ulevitch (Scripps Clinic and Research Foundation): Dr. Ulevitch is studying the mechanisms of endotoxin-induced hypotensive shock and tissue injury. He reports that in liver the hepatic macrophages exposed to bacterial endotoxin (LPS) induce a unique procoagulant activity acting directly on coagulation factor X. This activity may be of great importance in initiation of coagulative changes seen in liver exposed to LPS. Bacterial LPS was found to interact with plasma high density lipoproteins (HDL) forming a complex between LPS and HDL. These LPS-HDL complexes may be found in the circulation during gram negative bacterial septicemia; they have long half-lives and have the capacity to produce shock and disseminated intravascular coagulation. They are of great importance, therefore, to the septic patient who is under immunosuppression.

Streptococcal Diseases and Sequelae

The major streptococcal disease problem in the U.S. is still that of Group B streptococcal (GBS) infections; these continue to be a significant cause of neonatal morbidity and mortality. Group A infections and their principal sequelae of rheumatic valvular heart disease and acute glomerulonephritis are of lesser importance in the U.S., but remain as a significant problem in the developing countries.

Research Highlights

N01 AI 72539 R. M. Swenson (Temple University): Efforts of this contractor are directed towards development of a polysaccharide candidate vaccine for Group B streptococci (GBS) in neonates. The GBS types II and III vaccine materials showed good antibody levels in volunteers even after one year following the initial immunization. Candidate vaccines for GBS types Ia and Ib currently are under study for immunogenic activity. An ELISA test for antibody assay was developed; excellent linear results have been achieved with acute and convalescent human sera and sera from immunized volunteers. In studies with a neonatal rat model, antibody from immunized volunteers protected rats from lethal challenge infections of homologous GBS types.

AI 16674-02 D. Wennerstrom (University of Arkansas): The goal of this study is to evaluate the biochemical and cellular determinants of GBS pathogenesis in lung tissue. Mice were used as an appropriate animal model of human early onset GBS disease. The data showed that virulent GBS type Ia caused a reduction in the amount of pulmonary surfactant lipid. Secretion of nascent surfactant from tissue was inhibited by GBS infection, and not as a consequence of surfactant synthesis inhibition. This suggests a role for altered metabolism in decreasing the surfactant during infection. Purified lipoteichoic acid (LTA) of GBS type Ia cells instilled into mouse lungs caused death, with a calculated LD_{50} of 500 micrograms; a marked respiratory distress and pulmonary hemorrhage was observed. This is similar to results seen in human neonatal infections.

Other Disease Problems

There is a wide array of other disease problems that merit support by the Institute. Certain infections, such as Legionnaires' Disease and Toxic Shock Syndrome, have emerged only recently as significant disease areas. Despite the widespread use of antibiotics, many other bacterial infections remain as important health problems.

AI 17254-02 M. Horwitz (Rockefeller University): Dr. Horwitz wishes to gain a better understanding of the role of the immune system in Legionnaires' Disease. He has demonstrated that specific cell-mediated immunity to the organism does develop in patients. Taken together with earlier findings that imply a limited role for humoral immunity, this indicates that cell-mediated immunity may be significant in host defense against Legionella. Other data suggest that patients with the disease, under treatment with erythromycin and rifampin, may still require host defenses to eliminate the Legionella organism. Studies on the clinical course of the disease in immunocompromised patients point out that inadequate host defenses may result in relapse following cessation of antibiotic therapy.

AI 18359-02 P. Schlievert (University of Minnesota): This investigator is evaluating the association of staphylococcal pyrogenic exotoxin, type C (PE-C) with toxic shock syndrome (TSS). All Staphylococcus aureus isolates from TSS cases expressed the PE-C, thus the PE was specific for TSS isolates. Of the non-TSS staphylococcal isolates, 15% were vaginal isolates that also expressed PE; a majority of TSS isolates belonged to phage group I. Phage group II strains, which often make exfoliative toxin, failed to express PE. Examination of strains from individuals without a history of TSS (from 1960-1970) showed they also failed to make PE. In 1971, however, 10% of strains tested were positive for PE, and by 1976, the percent positive reached a maximum level. The data suggest that the TSS associated S. aureus is either a newly emergent strain or was imported into the U.S. in the early or mid-1970's. It should be noted that Super Absorbing tampons were not available on U.S. markets when the TSS-associated strain was emerging.

AI 17059-02 J. Oliver (University of North Carolina): This project is an investigation of the ecology of a recently identified halophilic pathogenic organism, Vibrio vulnificans. The species was identified only 5 years ago; it is highly invasive and associated with a high incidence of septicemia and high mortality (7-10%). The organism, found in a marine environment, can enter the body by ingestion or through breaks in the skin. It rapidly causes disease; symptoms are chills, hypotension and skin lesions on the extremities that subsequently become edematous. Cutaneous infections frequently result in limb amputation. To date most of the isolations of V. vulnificans have been from the mid-Atlantic coastal areas. Currently, studies are directed toward production of extracellular products that may contribute to the remarkable invasiveness of this organism. In a mouse model, evidence is strong that iron may play a major role in pathogenesis; it may be the limiting factor on the ability of the organism to survive and grow in mammalian sera.

AI 16993-02 D. Feingold (University of Pittsburgh): Data from this project on mucoid characteristics of Pseudomonas from cystic fibrosis (CF) patients show that strains of Pseudomonas colonizing the CF patients are more heterogeneous than previously thought. Multiple mucoid and non-mucoid strains of the organism were isolated from single samples of CF sputum. Detailed analyses of the isolates revealed that the strains were extremely heterogeneous by many different parameters. These data suggest that the presence of multiple strains in CF patients may further complicate efforts to eliminate the organism. Mucoid strains may share some common property related to their colonizing ability, but this property is probably unrelated to polysaccharide structure or stability of the mucoid phenotype.

Leprosy

It is widely believed that leprosy is a highly infectious disease, hence the term "leper" commonly refers to an individual who is shunned by society because of the disease. In fact, the disease is only mildly contagious and as many as 90 percent of the world's population may be immune to it. It is known that individuals whose disease is controlled by drugs are completely noninfectious. It is also believed that leprosy has been largely controlled; this may be true in the United States, where there are at most some 5,000 cases, but it is not true in the underdeveloped countries of the world. It is estimated that there are about 12 million cases world-wide, with 3.5 million in India alone; the prevalence in some small communities may be as high as 7 percent. The new findings of resistance to the drug dapsone by the leprosy bacillus are worrisome and will become increasingly so as the many infections now in the incubation period reach the stage of overt clinical disease.

Research Highlights

N01 AI 02625 P. J. Brennan (National Jewish Hospital, Denver): Dr. Brennan has continued to characterize the species specific glycolipid antigens of *Mycobacterium leprae*. He has now completely sequenced the trisaccharides in both glycolipid I and II antigens. Both of these oligosaccharides are attached to a common phenolic lipid core. He has shown by radioimmunoassay that the pure phenolic glycolipid I reacts specifically with sera from lepromatous patients and does not react with sera from people with other mycobacterial infections. The demonstration that the glycolipid is serologically active when highly pure suggests the possibility of a useful diagnostic tool for this material.

AI 0821414 J. H. Peters (Stanford Research Institute): Dr. Peters has developed highly specific and sensitive liquid chromatography analyses for measuring, in biological fluids, concentrations of the drugs dapsone, rifampin, and clofazimine used in leprosy therapy. Since the collection of saliva is a noninvasive technique, it was anticipated that salivary and plasma drug concentrations would be proportional. The data revealed that salivary levels of dapsone was indeed proportional to the patient's plasma levels of this drug. The method was not sensitive enough however, to detect salivary levels of clofazimine, and salivary levels of rifampin could not be correlated with patient's plasma levels. Nevertheless, since dapsone is the most commonly used antileprotic drug worldwide, Dr. Peters' methods may prove of significant value in studies of the development of dapsone drug resistance in highly endemic areas of the world.

Tuberculosis

Three million people still die world-wide from tuberculosis every year. Four or five million new cases of acute, highly contagious pulmonary tuberculosis emerge each year, plus an equal number of new cases of less serious forms, which are nevertheless dangerous from a public health standpoint. Without treatment, new cases remain contagious for about two years before either death intervenes or a more or less precarious cure occurs spontaneously. This makes a total at any given time throughout the world of at least ten million contagious cases that are capable of spreading infection. Most of the victims live in developing countries, where the majority of cases are rarely diagnosed. The most powerful weapon in tuberculosis control is the combination of casefinding and chemotherapy. In the U. S., the number of reported cases of tuberculosis remains at approximately 28,000 per year.

Research Highlights

AI 0840113 M. B. Goren (National Jewish Hospital, Denver): Studies of "cord factor" (trehalose dimycolate) of *M. tuberculosis* and its toxic properties have shown that an interstitial and hemorrhagic pneumonitis is induced at early stages of cord factor intoxication in mice. Identical lesions are produced by entirely synthetic cord factor analogs and pseudo cord factors. Participation of T-cells in mediation of cord factor toxicity is implicit in the observation that nude nu/nu mice show considerable resistance to the glycolipid, whereas nu/+ mice are as susceptible as their homozygous normal counterparts. Dr. Goren is also probing the role of *surface* in the biological activities of cord factor. At critical concentrations, monolayers of the glycolipid form on hydrophobic polystyrene beads; at higher concentrations, the beads aggregate in a fashion that resembles the *cording* of virulent tubercle bacilli. This is the first evidence that cord factor may indeed be involved in this phenomenon of aggregation.

AI 1510202 T. M. Daniel (Case Western Reserve University): Dr. Daniel has applied physiochemical and affinity chromatography fractionation procedures to *M. tuberculosis* PPD and has been able to identify and characterize several of the major antigenic components of this product. Mycobacterial arabinomannan was identified by crossed immunoelectrophoresis as a major component in all of the PPDs studied. It was determined that PPD contains multiple antigens and a large amount of apparently nonantigenic, denatured protein. Antigen 7, with a molecular weight of approximately 8,000 daltons, predominates among those protein and peptide antigens of *M. tuberculosis* that are not denatured in PPD preparation.

Mycology

It is extremely important that fungal infections be considered in the differential diagnosis of infectious processes in all immunocompromised patients. The etiologic agents of the majority of opportunistic fungal infections are ubiquitous in the environment and often comprise a portion of the normal flora of the patient or of the health care team. Rapid identification of the isolated fungi is important to determine if they are merely contaminants associated with the clinical specimens, or if they are contributing to the disease process. The distinction between pathogenic potential and opportunistic contaminant is only relative and must be applied to the individual patient with caution. To establish the etiology of an opportunistic infection, specific criteria must be fulfilled. The general belief among investigators in this field is that many more people are suffering from mycotic infections than are being diagnosed.

Research Highlights

AI 0701507 G. S. Kobayashi (Washington University, St. Louis, Missouri): Dr. Kobayashi has been developing a method to generate mutants of *Histoplasma capsulatum* that are unable to undergo transitions from the yeast growth phase to the hyphal phase. Mutants have been obtained by a method that includes treatment with ethyl methanosulfonate and subculture on special medium. Both auxotrophic and morphologic mutants were obtained. The mutant yeast phenotypes were of three categories: cells growing at 37°C but unable to undergo normal morphogenesis to hyphae at 25°C; cells growing as hyphae but unable to transform to the yeast phase at 37°C; cells exhibiting a cold-sensitive auxotrophy. One mutant strain in this category was shown to be deficient in nitrogen metabolism, with a probable block in asparagine metabolism. These mutagenizing methods appear to be useful for generating and selecting specific H. capsulatum mutants for basic studies of its physiology and metabolism.

AI 1622803 G. Medoff (Washington University, St. Louis, Missouri): Previously, Dr. Medoff had shown that cysteine stimulates oxygen consumption in the yeast phase but not the mycelial phase of Histoplasma capsulatum. He has now extended this work and shown that the cysteine stimulation of oxygen consumption in the yeast is due to a cytosolic cysteine oxidase activity which is present only in yeast phase cells. Several distinct stages were apparent when cellular respiration, cysteine levels and cysteine oxidase activity were monitored during the mycelial to yeast transition. One stage was characterized by a progressive decrease in respiration and intracellular concentrations of cysteine. This was followed by cessation of respiration and a sharp decline in cysteine levels. Completion of the morphological mycelial-yeast transition required exogenous cysteine; cellular mitochondria showed no respiration in the absence of cysteine. In the final stage, morphological transition proceeded to completion to the yeast phase with increased respiration and intracellular levels of free cysteine, characteristic of the yeast phase. The inhibition of mitochondrial respiration in the mycelial phase and its activation by cysteine in the dormant stage requires clarification. These studies ultimately may aid in understanding the pathogenesis of H. capsulatum infection.

VIROLOGY

Introduction: The Virology Program of the Bacteriology and Virology Branch continues to support both free-ranging research on the biology of viruses and research focused on pathogenesis of specific viral diseases. The new technologies, such as recombinant DNA cloning, RNA/DNA hybridization, and monoclonal antibodies, have hastened progress in many areas and opened up some completely new approaches to the study of viruses and the host response to viral infection at the molecular level.

Genital Herpes: Following recommendations submitted by the Virology Task Force in 1978, the Sexually-Transmitted Diseases Study Group in 1980 and two advisory workshops on prevention and treatment of genital herpes infections, the NIAID initiated efforts to increase research directed toward the acquisition of new and definitive information on the epidemiology and natural history of genital herpes. Applications for program project grants were reviewed by NIAID following announced solicitations; it is anticipated that at least one of these Program Projects will be funded in FY' 82.

Research on Viral Diseases

R01 AI 17069-03 S.P. Adler, (Medical College of Virginia): Dr. Adler has been conducting epidemiological studies on acquired cytomegalovirus (CMV) infections. CMV was cultured from 119 newborns admitted as patients after four weeks of age. At least five of the infected infants were born to CMV seronegative mothers. Infected, low birthweight infants received blood from 8 or more donors prior to CMV positive cultures and an average of 6.6 of these donors were CMV antibody positive. Uninfected low birthweight infants received blood from an average of only 3.3 CMV antibody positive donors. Thus, there was a highly significant correlation between the acquisition of CMV and the numbers of different blood donors for each infant, low birthweight and seronegative mothers. Clinically there appeared to be a marked impact of acquired CMV infections in these infants. These investigations indicate that transfusion acquisition is the primary mode of infection in low birthweight infants, so that the entire problem of blood transfusion and CMV infections needs careful review.

R01 AI 17057-03 R. T. Schooley (Massachusetts General Hospital): The cell mediated immune response to Epstein-Barr (EB) virus that characterizes the clinical picture of infectious mononucleosis is being investigated by Dr. Schooley to determine the regulating factors of B-lymphocyte activation by T-lymphocytes. Using highly specific monoclonal antibody reagents to T-cell idiotypes, the investigators found that cytotoxic T-lymphocytes emerge from the reaction between subset-specific T-cell and the transformed B-lymphocytes. The specific subset of T-cells active in this reaction may vary with the individual, but for optimal development of cytotoxic T-lymphocytes a collaborative interaction between two subsets (OKT4⊕ and OKT8⊕) appear to be important. These observations correlate with *in vivo* observations in renal allograft recipients treated for rejection with OKT3 antibody. They observed a very high rate of EBV reactivation and a significant amount of CMV-induced morbidity in this group of OKT3 treated patients. These studies suggest that great care should be exercised to monitor virus-induced morbidity in treatment regimens utilizing monoclonal antibodies to T-lymphocyte subsets.

R01 AI/NS 06246-17 J. G. Stevens, (University of California, Los Angeles): A portion of this study deals with Marek's disease (MD) of chickens, one that is not only of considerable economic importance, but also is useful as a model for the study of herpesviruses that induce lymphoproliferative diseases. Evidence has accumulated that classic MD represents a natural model for the Guillain-Barre syndrome (GBS) that has been associated with human vaccinations for viral diseases. Dr. Stevens is attempting to define the basis of the demyelinating peripheral neuropathy induced by MD virus in chickens as a possible mechanism underlying GBS. Results to date suggest that a persistent cell-associated viremia disseminates to peripheral nerves via blood lymphocytes, resulting in a latent infection of non-myelating Schwann and satellite cells. As a consequence of the latent infection, viral induced antigen expressed in the cell membrane is detected by reactive lymphocytes. This cell-mediated immune response against the viral antigens may cause the lymphocytes to release lymphokines or enzymes that disrupt the integrity of a few myelin sheaths. Myelin derived from these sheaths, together with viral antigens which might act as adjuvants, could sensitize the host to myelin and set the stage of an autoimmune demyelinating process. Anti-myelin antibodies have been detected in some MD chicken nerves and in sera, and MD chickens have been shown to develop a delayed-type hypersensitivity to chicken peripheral nerves. Additional studies may elucidate more fully the contribution of the immune response to the pathogenesis of this disease.

R22 AI 17995-02 L. Rosen (University of Hawaii): Transovarial transmission (TOT) of all four serotypes of dengue virus was demonstrated by Dr. Rosen in *Aedes albopictus* mosquitoes. However, only serotype 1 of dengue was transovarially transmitted by the only urban vector for man, *Aedes aegypti*. Thus, TOT in the human vector does *not* appear to be an important factor for the persistence of dengue viruses in endemic areas, but TOT in other mosquito species may play a role in the survival of this important pathogen for man. In the course of these studies, Dr. Rosen demonstrated also that three strains of *Aedes triseriatus* mosquitoes from the continental United States were susceptible to oral infection with dengue type 1 virus. The finding that Ae. triseriatus can serve as a vector of dengue is important not only because of its possible role in the transmission of the virus to man in the United States, but also as an indication that the mosquito host range of dengue viruses is wider than previously suspected.

R01 AI 09476-13 D. Porter (University of California, Los Angeles): Aleutian disease virus (ADV) in mink is a model of immune complex disease caused by an infectious agent. This laboratory has studied the pathogenesis of this persistent viral infection which, in genetically susceptible mink, usually leads to a severe or fatal glomerulonephritis or arteritis. The putative agent has now been classified as a parvovirus, a DNA containing virus among the smallest of viruses characterized to date. The fully virulent, wild-type virus has been found to be temperature sensitive in vitro and is difficult to propagate in cell cultures, but it replicates rapidly and persistently in mink. A variant of the virus has now been found that is attenuated for mink and replicates well in cell culture. Infection of mink with the attenuated virus confers a partial immunity against challenge with fully virulent ADV, in that infection by the challenge virus is not prevented but no disease ensues. Although immunologic protection has been elicited by prior exposure to a related virus, antiserum does not neutralize the virus, an enigma that has not yet been explained.

R22 AI 03028-23 W. C. Reeves (University of California, Berkeley): The ability of mosquitoes to become infected and transmit viruses that are pathogenic for man has been the subject of study in this laboratory for over 20 years. Studies during the past year have revealed several important facts about strains of these vectors in the United States. Culex tarsalis is a vector for Western encephalitis (WE) and St. Louis encephalitis (SLE) viruses. It was demonstrated that high summer temperatures are detrimental to infecting this vector with WE virus, but the high temperature actually favors infection with SLE virus. Cx. tarsalis is a more efficient vector for SLE virus than Cx. quinquefasciatus; the virus is transmitted transovarially in this mosquito host, possibly explaining its ability to overwinter in endemic sites. Studies also revealed that transovarial transmission rates of California encephalitis (CE) virus in Aedes melaniman reached over 90% and the virus persisted for at least 8 months in infected eggs. It appears these viruses can persist unaltered for long periods in the vector without need for amplification through a vertebrate host. The information derived from these studies is important for developing strategems for control of these viral pathogens, which occasionally break out of their endemic state to cause serious epidemics.

Basic Studies on Viruses

R01 AI 08388-14 D. Baltimore (Massachusetts Institute of Technology): Investigators from this laboratory reported the successful production of a set of bacterial plasmids containing the complete genetic sequence of poliovirus type 1. The investigators have now made possible the cloning of all the components of viruses required to establish infections in mammalian cells. This unique and landmark accomplishment, however, falls short of in vitro synthesis of the complete virion particle because the assembly of the positive-sense RNA genome to the viral capsid still requires elements from the host cell. All positive-sense RNA viruses have genomes that are capable of infecting cells and initiating viral production without the aid of the viral capsid. By synthesizing the complete genetic sequence virologists can probe the intricacies of genetic modulation, gene function and regulation. In turn, much will be learned about the interaction between the virus and the host cell.

R01 AI 18272-01 A. Dasgupta (University of California, Los Angeles): Recently, Dr. Dasgupta has been able to demonstrate the synthesis of full-length poliovirus RNA (cRNA) that is complementary to the viral genome and serves as a template for the synthesis of progeny viral RNA. Two systems for this synthesis have been developed: 1) a system requiring host factors, and 2) a system independent of host factors. Both systems are capable of initiating cRNA synthesis without an added polio (U) primer which is usually required for *in vitro* systems. These results represent another step toward the complete *in vitro* synthesis of a virus that is pathogenic for man; they greatly enhance the means of elucidating the genetic mechanisms controlling complete synthesis of this virus. Dr. Dasgupta is planning to use highly specific (monoclonal) antibodies to identify, isolate and characterize the primer for the synthesis described.

R22 AI 16687-03 R. F. Ramig (Baylor University): Dr. Ramig has been studying replication and genetics of rotaviruses, a group of double-stranded, segmented RNA viruses that infect the intestinal tract and cause severe diarrhea in susceptible animals and man. The SA11 strain is infectious and pathogenic for nonhuman primates but not man and has served as a model for the human virus. The SA11 virus is easily propagated in cell culture whereas, until recently, the human strains were not. Using temperature-sensitive mutants to follow the recombination of genes controlling replication, Dr. Ramig has been able to define five recombination groups that range from complete control to no control over the replication process. Thus, recombination of genes controlling replication does occur during mixed infections. The genetic lesion in these mutants strongly inhibit both virus yield (replication) and plaque formation in cell culture (pathogenesis).

CLINICAL AND EPIDEMIOLOGICAL STUDIES BRANCH

The CESB serves as the Institute's focus for the development and processing of Investigational New Drug Applications, and maintains liaison with the NIH Human Research Review Panel and the NIAID-Clinical Research Subpanel. It provides and monitors a closed clinical facility for Institute volunteer studies, and conducts, promotes and supports the development and evaluation of procedures for the diagnosis, prevention and treatment of infectious disease, particularly those procedures leading to improved patient care. The Epidemiology and Biometry (EB) Section conducts epidemiological research in infectious, allergic and immunological diseases, and analyzes data from various sources for trends in U.S. Vital Statistics in areas of interest to the Institute. The EB Section manages the Institute's program for mathematical and statistical studies, and provides biometrical consultation to other branches and laboratories.

Approximate Level of Support

Activity	Number	Amount
Epidemiology, Antibiotic Trials, Rapid Viral Diagnosis, Nutrition, Volunteer Facility		
Research Contracts	6	$1,797,870
Research Grants	6	$515,738
Total	12	$2,313,608
Diarrheal Diseases		
Research Contracts	6	$400,796
Research Grants	25	$1,992,262
Program Project Grants	1	$169,180
Training Grants	1	$29,069
Total	33	$2,591,307
Branch Total		
Research Contracts	12	$2,198,666
Research Grants	31	$2,508,000
Program Project Grants	1	$169,180
Training Grants	1	$29,069
Total	45	$4,904,915

Enteric Diseases Program

With the departure of Mrs. J. Smith, Dr. Richard Horton became the Assistant Project Officer to Dr. Robert Edelman for the Enteric Diseases Program. The Enteric Disease Unit established last year at the Fort Apache Indian Reservation under contract with Johns Hopkins University (Dr. R.B. Sack) continues to make good progress in research on diarrheal disease. This project focuses on the large Apache population (5500 individuals) living in or near Whiteriver, Arizona. A clinical laboratory at the Indian Health Service Hospital has been established for the identification of most known viral, bacterial and parasitic enteric pathogens. Special diagnostic procedures are performed at the Division of Geographic Medicine laboratory in Baltimore. Several studies are in progress: one study includes surveillance of 200 households for diarrheal disease. This population is visited every two weeks; stool and blood samples are collected periodically. A longitudinal study is underway in which newborns are being followed from birth to age three years to ascertain their clinical diarrhea experience according to etiologic agent. During a two year period it is estimated that at least 100 newborns will be entered. A variety of cross-sectional, clinical and laboratory investigations of out- and in-patients presenting with acute diarrhea at the Indian Hospital are underway. Such studies include the role of rotavirus in upper respiratory and ear infections, comparison of oral rehydration fluids, and antibiotic therapy of campylobacter and shigella enteritis.

The Enteric Disease Study Unit at the University of Texas in Houston (Dr. H. Dupont) is concluding its long term surveillance of diarrheal disease in day care centers. A rotavirus prevalence survey was conducted in 29 centers. A total of 312 specimens from asymptomatic children were tested by the ELISA method; 12.5% were rotavirus positive. Stools from 7 to 12 month old males yielded the highest percentage of rotavirus, though little difference was observed between males and females in other age groups. In another surveillance study, three centers were studied for the presence of Clostridium difficile (Cd) for 3 1/2 months during which time five outbreaks of diarrhea occurred in children less than two years of age. Stool specimens collected during these outbreaks and afterwards were tested for the presence of Cd and its toxin. Cd was observed in stools from 13 of 21 children with diarrhea in a median concentration of 10^5 organisms per gram of stool. Stools from 12 of the 13 culture-positive children contained cytotoxin. Five of these 12 children had received antibiotics from one to four weeks prior to diarrheal episodes. In contrast, only 7 of the 44 asymptomatic children submitted Cd-positive stool specimens. Four of 7 were also positive for toxin, and 2 of these 4 children had received antibiotics prior to specimen collection. The differences in rates of Cd excretion and the presence of toxin between children with and without diarrhea are statistically significant ($p = 0.005$ for both Cd and toxin comparisons).

Final results are now available from studies conducted at the Enteric Disease Unit at Children's Hospital National Medical Center, Washington, D.C. (Dr. R.H. Parrott) In the period from November 1977 through September 1980, 1788 subjects from various populations were enrolled. Of the enteric agents, human rotavirus (HRV) was by far the most commonly detected cause of gastroenteritis. Visualized adenoviruses, Salmonellae, 27 nm viruses and Shigellae were next in order. Toxigenic E. coli were not associated significantly with enteric illness. Yersinia enterocolitica and Campylobacter were detected, although infrequently. Giardia lamblia was detected in one pediatric patient. There were significantly more episodes of gastroenteritis in families which had more than two children in the household, when compared to those with fewer children. Thirty three families experienced infection with rotavirus. Three of these families experienced rotavirus infections in two seasons. However, only one member, a father, had proven reinfection; he was symptomatic on both occasions. In a separate study, eleven persons were followed specifically for HRV reinfection; five had evidence of asymptomatic reinfection as detected by complement-fixing antibody.

During the past year, two research contracts dealing with the Diarrheal Disease Program were terminated because of budget cuts. One of these was a service contract with Dr. J. Craig, State University of New York Downstate Medical Center who evaluated and measured cholera toxin, antitoxins, and toxoids by the rabbit skin permiability assay and mouse protection test. The second contract was with the Johns Hopkins University School of Medicine. Dr. Nathaniel Pierce focused on new oral immunization regimens to protect dogs against experimentally induced cholera. These studies showed the efficacy and safety of crude cholera toxin and procholeragenoid and have stimulated trials of these antigens in man.

The CESB continued to coordinate the activities of the Cholera Panel of the U.S.-Japan Cooperative Medical Science Program. The 17th Joint Conference on Cholera was held October 25-28, 1981, in Baltimore, Maryland.

Closed Volunteer Facility

The CESB currently supports a research contract with the University of Maryland to maintain the Center for Vaccine Development (CVD) where volunteers are studied before and after they are challenged with potentially transmissible infectious agents and candidate vaccines. During the past year, the contractor performed 21 in-patient studies (290 volunteers) and 6 out-patient studies (234 volunteers). These studies were carried out under 9 active protocols approved by the Institutional Review Boards of the University of Maryland and NIAID. Included were Escherichia coli diarrhea (6 studies), cholera (5 studies), viral diarrhea (1 study), influenza (8 studies), Rocky Mountain Spotted Fever (2 studies), oral typhoid vaccine (1 study), recombinant DNA risk assessment (2 studies), and campylobacter (2 studies). Highlights of these studies are summarized below.

Texas Star-SR, a laboratory-derived mutant of Vibrio cholerae El Tor Ogawa which produces B subunit but not A subunit of cholera toxin, was fed to 68 healthy volunteers in graded doses. Side effects (loose stools, vomiting, abdominal cramps) were infrequent and mild when they did occur. The strain was genetically stable and did not secrete toxin in volunteers. Seroconversions of vibriocidal antibody occurred in 93% of vaccinees. Texas Star stimulated protective immunity in volunteers against challenge with virulent homologous El Tor Ogawa and heterologous El Tor Inaba serotypes. The Texas Star-SR provides an important prototype for an oral, attenuated V. cholerae vaccine. Dr. James Kaper (University of Maryland) has successfully cloned the genes encoding the enterotoxin from two V. cholerae strains in E. coli. The DNA sequence data and the construction of specific subunit mutants by Dr. Kaper and others will allow the development of attenuated, oral V. cholerae vaccine candidates which are more precisely defined and designed in their genetic and phenotypic characteristics than is Texas Star-SR.

Ty21a, a stable attenuated mutant of Salmonella typhi, is a safe, protective, oral vaccine when taken after neutralization of gastric acidity by $NaHCO_3$. Volunteers fed 1, 2 and 3 doses of enteric-coated vaccine were compared with those fed 1 and 3 doses of vaccine with $NaHCO_3$. The number of antibody conversions and anti O antibody titers were identical in each group. These results provided the basis for a field trial of Ty 21a vaccine in 90,000 Chilian school children.

Clinical and laboratory investigations were carried out to develop vaccines that can stimulate anti-adhesion (antipili) immunity against diarrhea caused by enterotoxigenic E. coli (ETEC). A parenteral E. coli vaccine consisting of purified type 1 somatic pili was tested in 100 volunteers. Taken as a whole, five challenge studies done during 1980-1982 failed to show a consistent protective effect of the vaccine. Following successful animal studies, a naturally occurring, non-toxic E. coli strain bearing colonization factor antigen II (CFA/II) pili was fed to 19 volunteers as an oral vaccine. Two persons developed diarrhea presumably as a consequence of colonization of the small intestine. The local and systemic antibody responses of these volunteers are pending. In collaboration with the Walter Reed Army Institute of Research, purified CFA/II pili antigen has been prepared for phase I tests as an oral vaccine in volunteers.

Skin tests as a measure of cell-mediated immunity to Rickettsia rickettsii were applied to the forearms of volunteers who participated in the Rocky Mountain Spotted Fever Vaccine Trial conducted 13 to 15 months earlier. The formalin-inactivated R. rickettsii vaccine produced by the U.S. Army was used as the skin test antigen. Only one positive delayed skin test reaction (DCH) occurred among the 16 controls who had never been exposed to R. rickettsii. By contrast all 17 individuals who had been challenged with R. rickettsii had DCH. In addition, seven (88%) of the 8 unchallenged vaccinees who had IFA antibody rises after vaccination, and 4 of 8 (50%) of such vaccinees who never had antibody rises, had positive DCH. Thus, the results of the RMSF skin testing appear promising and may be our best test for measuring CMI to R. rickettsii infection in man.

During the winter of 1981-1982, the CVD in collaboration with the LID, NIAID, conducted a large influenza vaccine study at the College Park Campus of the University of Maryland. Fifeteen-hundred students were interviewed, 337 were screened for seroeligibility, 104 had histories and physical examinations; 79 student volunteers were finally administered influenza A/Washington, D.C. 897/80 (H3N2) cold adapted (Ca) reassortant vaccine intranasally or commercially-available inactivated influenza subvision vaccine intramuscularly. The results showed the Ca vaccine was suitably attenuated for adults. In-patient efficacy studies of these vaccinees using Influenza A/Washington/897/80 (H3N2) wild-type virus challenge were conducted. Evaluation of illness responses, antibody responses and viral titers are in progress.

In a series of four recombinant DNA risk assessment studies in volunteers, Dr. Levine and co-workers clearly established for the first time the safety of biological containment utilizing poorly mobilizable cloning vectors such as the plasmid, pBR 325.

Collaborative Clinical Trials of Antibiotic Therapy

During its fourth year of operation, the contract with the University of Alabama to study improved therapy of cryptococcal meningitis and other systemic fungal diseases has provided important clinical and laboratory data. In Study 1, 146 patients with cryptococcal meningitis have been enrolled during the past four years. Among the 33 protocol adherent patients who received Regimen A (four weeks of combined amphotericin B and 5-flucytosine) seven relapses have occurred. By comparison, among 36 protocol adherent patients who received Regimen B (six weeks of combined drugs) five relapses have occurred. Because relapse rates are similar between the two treatment groups, a statistical evaluation will be made in the fall of 1982 to determine the probability of achieving a significant difference between the two regimens.

The contractor has completed Study 2 entitled "Phase II Trial of Ketoconazole in Five different Systemic Fungal Infections" and one manuscript entitled, "Treatment of Systemic Mycoses with Ketoconazole: Emphasis on Toxicity and Efficacy in 52 Patients" has been submitted for publication. Two other manuscripts dealing with laboratory aspects of this study are in preparation.

Good progress has been achieved in Study #3A entitled "Comparison of the Efficacy and Toxicity of Two Different Dosage Regimens of Oral Ketoconazole in Randomly Assigned Patients With Either Blastomycosis or Histoplasmosis." One hundred eleven patients were enrolled and the drug found to be quite efficacious in this trial. Ketoconazole has been implicated as the cause of several different adverse effects. The two most common toxicities have been nausea and/or vomiting (20% of patients) and skin rash (14.3% of patients). Miscellaneous toxicities have been observed in 20 patients. The most important of these side effects have been gynecomastia--2 patients, exaggerated premenstrual edema--1, and impotence and/or decrease in libido--2.

Eighty-four patients have been enrolled into Study #3B entitled "Comparison of the Efficacy and Toxicity of Two Different Dosage Regimens of Oral Ketoconazole in Randomly Assigned Patients With Coccidioidomycosis, Including a Provision for Ascending Doses of Ketoconazole." Thirty-three patients have been randomized to Regimen A, or low dose ketoconazole (400 mg/day); 35 have been randomized to Regimen B or high dose ketoconazole (800 mg/day). Due to the higher doses of ketoconazole administered, more adverse effects have been observed: nausea and/or vomiting, 32% of patients; chills and/or fever 5.6%; alopecia 6.9%; and gynecomastia 8.3%. Miscellaneous toxicities have been encountered in ten patients; the most important of these have been decrease in libido--1; azospermeria--1, and decrease sperm count and motility--1. In two patients, ketoconazole was judged to be a treatment failure because of the side effects.

Eight patients have been enrolled into Study #3C entitled "Pharmacokinetic and Toxicity of Various Doses of Ketoconazole in Coccidiodal Meningitis." Ketoconazole was shown in this study to reduce the blood levels of testosterone and other ketosteroids. Pending the results of more thorough hormonal studies by Dr. David Stevens (Santa Clara Valley Medical Center) to determine the effect of high ketoconazole dose and duration of therapy on the levels of cortisol and testosterone, there will be a constraint on enrolling patients into this study.

The contract with Dr. V. Schauf (University of Illinois) was phased out in July, 1982, one year earlier than orginally planned. This contract supported a placebo-controlled, double blind trial of penicillin in preventing early onset Group B Streptococcal (GBS) sepsis in the newborn. During the three years of the contract, project personnel enrolled over 2800 consenting women and randomized their newborn infants to receive intramuscularly, 100,000 units aqueous penicillin G or saline placebo immediately after delivery. The enrollment of mother-infant pairs was too slow to provide for the projected 10,000 subjects needed to successfully complete the study within the contracted time frame of four years. Although the total number of infants with GBS infection was too small to allow for meaningful statistical analysis, the contractor observed that penicillin was 100% effective compared to placebo for preventing early-onset disease, and 60% effective for preventing early plus late onset disease. Prophylactic penicillin significantly reduced GBS colonization of infants at discharge who were born of GBS colonized mothers.

The use of penicillin prophylaxis had no apparent effect on other perinatal infectious morbidity. Infants with clinical indications of invasive infection leading to performance of blood culture (11.2% penicillin vs 12.5% placebo), lumbar puncture (5.8% penicillin vs 6.3% placebo) and/or use of antimicrobial therapy were distributed evenly between penicillin and placebo groups. The yield from bacterial cultures of blood or CSF was similar in the two groups except that streptococci were isolated only in the placebo group. Mortality was also similar in the two groups.

Penicillin was well tolerated by the neonates. Reactions to the injection were mild, infrequent, and similar in placebo and penicillin recipients. There was no immediate hypersensitivity to penicillin. Two hundred and fifty-one parents of 1-2 year old children contacted by telephone agreed to be interviewed concerning subsequent use of antibiotics and possible allergic reactions in their infants. Strikingly, over one-half of the children in each group had been treated subsequently, at least once, with a beta-lactam antibiotic. None had experienced urticaria or respiratory distress. No immediate hypersensitivity reactions had occurred in either group. Rash consistent with penicillin allergy was present after subsequent beta-lactam administration in similar numbers of infants in both penicillin and placebo groups. Total allergic manifestations appeared somewhat more frequently in placebo recipients. Approximately 1/3 of each group had some family history of allergy. An additional 150 parents will be interviewed, and these data will be included in the final evaluation.

Final work will include studies on penicillin antibodies. Coded sera from over 100 of the infants whose parents provided data concerning beta-lactam exposure have been sent to Dr. F. Atkinson, Good Samaritan Hospital, Baltimore. These sera will be analyzed for IgG and IgE antibodies to the penicillin major determinant by RAST.

Nutrition, Infection and Immunity

The goal of this program is to promote reserach on the interaction of malnutrition, infection and immunity. Specifically, our interests focus on the modulating effect of specific nutrients on immune function and microbial virulence, on mechanisms of food allergies and immune response to ingested antigens, and on the interaction of nutrition and infection in the tropical environment and in American hospitals.

In FY 1982 the NIAID Program in Nutrition, Infection and Immunity consisted of 24 grants, subprojects of two contracts, one Research Career Development Award, and one intramural project on Food Allergy. Two grants were awarded for proposals in response to the RFA, Mechanisms of Food Allergy. The extramural program was monitored by at least four project officers in the IAID and MID programs, in addition to the CESB.

Rapid Viral Diagnosis

The three contracts in this program were advertised for recompetition this year. Seventeen responses were received. Of these, six were judged to be in the competitive range, but only two contracts were awarded for the next three years because of budget cut-backs. Highlights of the grant and contract programs during the past year include the following:

Dr. Nathalie Schmidt (California State Laboratory of Public Health) found that in contrast to what others have reported, antibodies to the early non-structural antigens of VZV and herpes simplex types 1 and 2 are not indicative of active infection; these antibodies to early antigens are of no more diagnostic significance than antibody responses to late antigens. She is using VZV monoclonal antibodies conjugated with fluorescein to identify VZV isolates and to identify

VZV antigen directly in lesion smears. This method, using direct immunofluorescence, is more sensitive for VZV detection than is cell culture. The VZV antibodies were very sensitive to the high pH and fluorescein concentrations used for standard conjugation procedures, but addition of dimethyl sulfoxide stabilized the antibodies during conjugation; this is an important finding since the homogeneity of monoclonal antibodies often is associated with increased lability.

Dr. Yolken (Johns Hopkins) has developed a rapid multiple-determinant enzyme immunoassay (EIA) for the detection of human rotavirus in clinical specimens. This rapid solid-phase EIA makes use of simultaneous binding of enzyme-labeled and solid-phase antibodies at immunologically distinct antigenic sites. The test, which does not involve prolonged incubations or multiple washing steps, is performed in less than 40 minutes. He used it to accurately detect rotavirus antigen in 45 stool specimens positive for rotavirus. No false-positive reactions were noted provided that appropriate control reactions were performed. He devised an analogous assay for adenovirus which can detect as little adenovirus antigen in 40 minutes as the standard assay which requires 4 hours.

Using his EIA panel for diarrheal agents, Dr. Yolken found that 7 of 14 bone marrow transplant recipients were infected with Coxsackie A1 during an outbreak of diarrhea. Following this outbreak a prospective study was performed of infections with common gastrointestinal agents in patients undergoing bone marrow transplantation during a 10-month period. A total of 31 (40%) of 78 patients were infected with one or more of the enteric pathogens being tested. Infection was significantly correlated with the occurrence of diarrhea and abdominal cramps. The mortality rate of patients infected with one of the gastrointestinal pathogens was 55%, which was significantly higher than the 13% mortality noted in the non-infected patients.

Dr. Richman and his collaborators (University of California, San Diego) have continued developmental studies of their immunofiltration technique which uses non-specific antigen immobilization rather than capture antibodies. They have developed sensitive and specific antigen and antibody assays for influenza viruses, herpes viruses, and Ebola-Marburg viruses. The assay has been converted from radiolabeled to enzyme immunoassay with a colorigenic substrate, permiting visual reading and shortening of the assay time to two hours. The assay continues to be refined, and is now to the stage of being evaluated with clinical specimens. It can detect with 100% specificity viral antigens in 95% of culture-positive eye swabs from rabbits with herpes simplex kerato-conjunctivitis.

Epidemiology and Biometry Section

The Epidemiology and Biometry Section (EBS) has continued to pursue epidemiologic research in infectious and immunologic diseases. In addition to Dr. Kaslow and Dr. Blackwelder, Dr. Saah has been fully integrated into the unit's program. Other part time and temporary professionals have been engaged in selected epidemiologic activities. A second Epidemiology Associate, Dr. Anne Bailowitz, is now enrolled in the training program at Johns Hopkins School of Hygiene.

Infectious Diseases

Work has continued with Dr. LaMontagne and others on influenza morbidity and mortality at the Jewish Home and Hospital for the Aged in New York City. This work was expanded in 1981 from the retrospective study of the apparent efficacy of influenza vaccine against pneumonia mortality to include a prospective, non-randomized clinical trial of influenza vaccine in this aged population. A preliminary report of results will be presented at a meeting of the American Public Health Association (APHA). In a related project, Dr. Blackwelder continued to work with Dr. John LaMontagne, DAB, on data analysis for the 1978 Russian influenza vaccine trial.

The EBS has become deeply involved in the regional cooperation effort between Egypt and Israel. The project is funded by USAID and administered by NIAID. Dr. Fred Feinsod will be stationed in Egypt for two years as a visiting scientist from EBS, NIAID. He and Drs. Saah and Kaslow have collaborated with the Egyptian scientists at the Ain Shams University in Cairo in preparing a protocol to study Rift Valley fever in man and animals in Egypt. American, Israeli and Egyptian scientists met in Stockholm in June 1982 to discuss their work to date and to plan for future collaborative work. Malaria and leishmaniasis have also been targeted for study by the regional cooperative effort, and the EBS is taking a consultative role in these studies.

Dr. Willy Burgdorfer at the Rocky Mountain Laboratory recently isolated a spirochete from the tick vector of Lyme disease; this bacterium is thought to be the etiological agent. To help confirm the organism's role in producing Lyme disease, a series of studies, including a prevalence serosurvey in a Long Island community, have been undertaken in collaboration with local investigators. A protocol for the study of sera and other tissue from active cases has also been developed. Ecologic studies on the vector and animal hosts are underway with collaborators in Wisconsin as well.

The unit has begun a descriptive study of infections and malignancies in the 2500 patients enrolled in the Kidney Transplant Histocompatibility Study (KTHS). The focus is on demographic and clinical factors and an effort will be made to evaluate the role of splenctomy in infection in transplanted patients. Depending on the number of cases of Karposi's sarcoma and other malignancies found, the study may also add to the growing body of information about those cancers and immunosuppression.

Along the same lines, a questionnaire on selected risk factors has been prepared for administration to acquired severe immunodeficiency syndrome patients admitted to the NIH Clinical Center. An effort is also being made to search army hospital records for evidence in that population of occurrence of any conditions related to the immunodeficiency epidemic.

Maternal infection during pregnancy is thought to be one of several factors that can influence birthweight. Preliminary work is underway to assess the specific role of genitourinary tract infection and its possible relationship to prematurity. Data from the Collaborative Perinatal Project are being reanalyzed, and previously unanalyzed data on neonatal sepsis may answer related questions about infection in mothers and infants.

The EBS has a commentary in press in JAMA on the treatment of otitis media. A recently published report of a clinical trial concluded that there was no difference in outcome whether or not children received antibiotics. Despite major flaws, the trial received wide, uncritical citation; the EBS commentary provides a more critical review.

Immunologic Diseases

A protocol to study prenatal factors in the development of childhood asthma was submitted to and accepted by NINCDS. Data from the Collaborative Perinatal Project will be abstracted and analyzed on about 2000 children identified as having asthma.

Studies on environmental and genetic risk factors for systemic lupus erythematosis are in the early stages.

Interest in HLA and disease relationships is also being pursued by Dr. Blackwelder in collaboration with Dr. Robert Elston of Louisiana State University. Their methodologic research on tests of genetic linkage using sibship data promises to guide investigators in study design and to improve the power of analysis of family studies.

Biometric and Consultation Research

Dr. William Blackwelder served as consultant to NIAID project officers and as a member of data monitoring committees for collaborative clinical trials on: 12) comparison of two durations of therapy with amphotericin B and 5-flucytosine in cryptococcal meningitis, 2) efficacy of inactivated hepatitis B vaccine in patient and staff of dialysis units, 3) efficacy of adenine arabinoside monophosphate alone and combined with human leukocyte interferon in chronic hepatitis B infection, 4) and efficacy of antiviral substnces in encephalitis and neonatal herpes infections.

Dr. Blackwelder and Ms. Marie Chang prepared a series of graphs indicating appropriate sample sizes for studies on equality of therapies for a presentation at a meeting of the Society for Clinical Trials. Dr. Blackwelder collaborated with researchers at Temple University in analyzing hematological data on volunteers challenged at the University of Maryland with the Rocky Mountain Spotted Fever rickettsia.

Computer tapes from the National Center for Health Statistics (NCHS) containing all conditions listed on the death certificate are now available for several recent years, and preparations are being made for their analysis with respect to infectious and immunologic conditions contributing to death. Preliminary arrangements have also been made with NCHS to obtain death certificates on recent deaths from Reye syndrome to examine the geographic distribution of fatal cases.

Other Activities

The Investigational New Drug Applications (INDAs) for the Microbiology and Infectious Diseases Program are filed and maintained by the CESB. Currently 22 active INDAs are on file with the Bureau of Biologics, Food and Drug Administration (FDA), and six with the Bureau of Drugs, FDA. During the past year, new INDAs have been filed for *E. coli* K100 Capsular Polysaccharide which is cross-reactive with the polyribose phosphate of *H. influenzae* type B, Type III group streptococcus polysaccharide vaccine, and cholera toxin.

The branch staff served as Institute representatives on many Trans-NIH committees: Dr. Horton--Digestive Diseases, Diabetes, and Cystic Fibrosis; Dr. Kaslow--Epidemiology, Diabetes; Dr. Edelman--Nutrition, Clinical Trials; Mrs. Mattheis--Review Policy Committee on Contracts Involving Human Subjects.

Dr. Edelman was appointed as Institute representative and ex-officio member of the National Digestive Diseases Advisory Board. He continued to serve on the Human Ethics Committees (Institutional Review Boards) of the Walter Reed Army Institute of Research and the NCI-Frederick Cancer Research Center. As Clinical Associate Professor of Medicine, Dr. Edelman served as a preceptor in Clinical Medicine Uniformed Services University of the Health Sciences.

DEVELOPMENT AND APPLICATIONS BRANCH

Consistent with its name, the primary objective of the Development and Applications Branch is the translation of new information derived from basic research into methodologies appropriate for the control or prevention of infectious disease areas designated as high priority for extramural support by the Institute. Applied research activities include identification of important infectious disease problems with potential for control or prevention through immunization or utilization of antivirals or hyperimmune sera, development of appropriate vaccines, antivirals, and other control measures, design and support of appropriate clinical trials for evaluation of control measures. In addition, basic research in related areas is supported within the Branch.

Approximate Level of Support

Activity		Number	Amount
Vaccine Evaluation Units			
Research Contracts		5	$ 1,263,443
	Subtotal	5	$ 1,263,443
Influenza			
Research Contracts		6	782,647
Interagency Agreements		2	50,440
Research Grants		27	2,988,093
Young Investigator Awards		2	97,978
Fellowships		1	17,040
	Subtotal	38	$ 3,936,198
Respiratory Diseases			
Research Contracts		1	0
Research Grants		6	525,000
	Subtotal	7	$ 525,000
Hepatitis			
Research Contracts		6	1,465,141
Interagency Agreements		3	212,693
Research Grants		9	819,275
Young Investigator Awards		2	75,725
Fellowships		2	39,932
	Subtotal	22	$ 2,612,766
Antiviral Substances			
Research Contracts		15	1,801,186
Research Grants		36	3,129,301
Career Awards		1	40,683
Fellowships		1	17,040
Training Grants		1	95,182
	Subtotal	54	$ 5,083,392

Bacterial Vaccines

Research Contracts	10	343,117
Research Grants	31	2,711,185
Young Investigator Awards	1	43,515
Career Awards	2	75,221
Fellowships	6	125,684
Subtotal	50	$ 3,298,722

Viral Vaccines

Research Contracts	1	0
Research Grants	8	770,379
Young Investigator Awards	1	54,962
Fellowships	1	19,040
Subtotal	11	$ 844,381

Branch Summary

Research Contracts	44	5,655,534*
Research Grants	117	10,943,233
Interagency Agreements	5	263,133
Young Investigator Awards	6	272,180
Fellowships	11	218,736
Career Awards	3	115,904
Training Grants	1	95,182
Totals	187	$17,563,902

*No FY82 funds are shown for 19 contracts which, although in effect during part or all of FY82, received funding in prior years.

Program Summary

Activities of the Development and Applications Branch include comprehensive research programs on influenza, other viral respiratory diseases, viral hepatitis, viral vaccines, bacterial vaccines and antiviral substances. Because these programs are designated as high priority for development of control measures, funding is provided through all mechanisms available to staff. The DAB carries out many of its objectives through directed research, and is responsible for the major portion of the contract activities within the Microbiology and Infectious Diseases Program.

In recent years, there have been severe reductions in contract funds within the Institute. This is of great concern to the Branch staff as a balance in mechanisms available to program is very important to permit break-throughs in basic research to be developed and applied to the clinic. Further reductions in resources for contract-supported applied research will adversely affect our ability to obtain "payoffs" from basic research programs.

In contrast to the epidemic winter of 1980-1981, this past winter saw only scattered localized outbreaks of influenza. Few influenza A viruses were isolated. The scattered outbreaks were due to H1N1 or influenza B viruses. Hence, planned clinical studies for evaluation of antiviral agents had to be postponed. However, some progress was made in evaluating rimantadine and ribavirin for the prevention and/or treatment of influenza. Advances have been made toward the development of attenuated vaccines. The cold-adapted influenza vaccine has been shown to be attenuated and genetically stable and human efficacy studies are underway. Progress has also been made on the development of vaccines by reassortment between avian and human viruses and by using recombinant DNA technology. Last year there was a reduction in the number of cases of Reye's Syndrome, probably related to the milder influenza season. Studies linking aspirin use to Reye's syndrome have led to the recommendation that it not be used to reduce fever in children with influenza or chickenpox.

The hepatitis B vaccine produced by NIAID was shown to be non-reactogenic and highly immunogenic in adults and children. This lot of vaccine is currently being used in the People's Republic of China to determine its effectiveness in preventing maternal-fetal transmission of HBV. Early analysis of data from the NIAID sponsored trial of Merck vaccine in patients and staff of hemodialysis centers has shown that the vaccine is highly protective in staff. Subtype cross-protection was seen in the staff population. Experiments continue to determine the potential of a woodchuck hepatitis virus (WHV) vaccine for prevention of disease and pevention of maternal-fetal transmission in humans. Establishment of a woodchuck experimental facility is critical to the future of these studies.

During the past year, the FDA approved an antiviral drug, Acyclovir (Zovirax) for use in treating primary genital herpesvirus infections and in preventing mucocutaneous herpes simplex virus infections in immunocompromised individuals. DAB clinical studies have not shown a significant effect of topical acyclovir in individuals suffering from herpes labialis. During the past year, vidarabine (adenine arabinoside) was shown effective in the treatment of varicella-zoster virus infections in the immunocompromised host. Licensure for its use in zoster infections in the immunocompromised host is imminent. Vidarabine was also demonstrated to be of value in treating chicken pox in immunocompromised patients when given early in the course of infection. Clinical studies to compare vidarabine and acyclovir in herpes encephalitis and neonatal herpes infections are in progress. Trials are also underway to determine the value of parenterally administered interferon in females with initial genital herpes infections and to compare the use of ara-AMP alone and a combination of interferon and ara-AMP in therapy of hepatitis B carriers.

The Bacterial Vaccines Program continues studies of pneumococcal vaccine in high risk patient populations. Trials of pneumococcal vaccine for the prevention of otitis media have shown, unfortunately, that the vaccine was of limited value in preventing overall pneumococcal otitis media. In those cases in which infants mount a good antibody response to a particular polysaccharide, type specific protection could be shown. Future efforts must be directed toward making polysaccharides which are immunogenic in infants. Numerous approaches are being used to develop a Haemophilus influenzae type b vaccine which will be immunogenic in infants. In addition to NIAID sponsored efforts, several drug companies are working to develop Haemophilus influenzae vaccines. Collaborative studies have begun with a promising new candidate vaccine produced by Lederle Laboratories. A combined protein-polysaccharide vaccine for group B meningococcal disease has shown potential in studies in adults, and infant studies are in progress. The new Army Rocky Mountain Spotted Fever (RMSF) vaccine was not effective in preventing disease in vaccinated humans challenged with RMSF agent, although the severity of the disease was significantly reduced. Plans are also underway to develop a new subcellular pertussis vaccine for human testing and to perform human testing on a subcellular vaccine developed by the Japanese.

Research indicating the role of fusion protein (F) in paramyxovirus spread in tissue cultures has provided new impetus for identifying such a component in parainfluenza 3 and RSV. Studies are underway to identify, isolate, and purify surface glycoproteins of para-3 for use as vaccines. Using monoclonal antibodies, considerable progress has been made in associating structure with function in RSV; an important step in developing a candidate vaccine.

Over 100 children and 20 adults have been vaccinated with an attenuated varicella vaccine to determine its value in protecting the immunosuppressed against varicella infections. The vaccine has been well tolerated and all vaccinees have had antibody responses.

Research Highlights

Influenza Program

The last year will be remembered as a relatively quiet one in the history of influenza. In contrast to the serious epidemic experienced in 1980-1981, this last winter was very mild. Influenza A viruses were infrequently isolated in the United States during the last winter and only scattered localized outbreaks were observed. These were mostly attributable to H1N1 or influenza B/Singapore/79-like viruses. It is potentially of great interest that influenza viruses were isolated from sporadic outbreaks late into the spring and early summer. These outbreaks occurred in nursing home populations. The epidemiologic events in the United States generally reflect the events in other parts of the globe. However, Japan experienced a severe influenza B epidemic, which was associated with substantial morbidity in school populations and the occurrence of Reye's syndrome.

Research on influenza and influenza viruses continues to result in significant developments. For example, important milestones in the development of attenuated influenza vaccines have been reached. The cold-adapted influenza vaccine has now been shown to be attenuated and genetically stable. These characteristics are important in a vaccine. Six reassortants containing the desired attenuating genes from the master strain A/Ann Arbor/6/60 have now been tested in over 2000 individuals. These vaccines have consistantly been immunogenic, genetically stable, and non-reactogenic. Efforts to establish the value of these vaccines in the face of natural challenge are underway. Alternative vaccines are also under development. Of particular interest are two attenuated vaccines. One of these is based on the generation of vaccine candidates from the genetic reassortment between a selected avian virus and relevant human strains. In addition, efforts to reconstruct an attenuated vaccine through recombinant DNA methodology have continued. The successful expression of several influenza genes from a series of SV-40 influenza DNA vectors has now been achieved. It is hoped that mutations can be introduced into these sequestered genes and that eventually a solidly stable master strain will result.

Progress has also been made in the development, testing and application of antiviral drugs to the prevention and treatment of influenza infections. Amantadine continues to be used for this purpose. Results from recent studies supported by the DAB have clearly shown the value of rimantadine for the prevention and/or treatment of influenza infections. A third antiviral drug, ribavirin, has also been shown to be effective in the treatment of severe influenza A and B infections when administered as an aerosol preparation.

Basic research on influenza viruses continues to be productive. New insights into the process of antigenic drift have been obtained through a careful analysis of the sequences of the hemagglutinin, neuraminidase, matrix, nucleocapsid and non-structural genes. These data, and monoclonal antibody analyses, have given clear evidence for the nature of these changes. The direct application of this information in the production of newer inactivated vaccines through the chemical synthesis of specific antigenic determinants is now possible. Work along these lines has been pursued during the last year. Basic research has also provided new techniques for diagnosis. The incorporation of sensitive ELISA methodology for the identification of viral proteins from the secretions of infected individuals is very encouraging. In addition, these methods are being used to assess antibody responses after infection or vaccination.

Research into the nature and variety of the immune response to influenza antigen has also produced significant new information with implications for vaccine development. Humoral and cellular immune responses have been demonstrated in reponse to immunologic challenge by influenza antigens. The contribution of these responses to immunity is still being analyzed, but cellular responses have been shown to be important in localizing infection and in the recovery from infection. Also significant is the growing realization that inactivated influenza antigens are relatively poor inducers of these cellular responses in unprimed individuals. Infection with wild-type or attenuated viruses seem to induce significant cellular immune responses.

This last year was a significant one in the history of Reye's syndrome. Four field studies indicated that aspirin use was temporally related to the development of Reye's syndrome. These studies resulted in the recommendation by the Public Health Service and the Food and Drug Administration that aspirin not be used for the treatment of fever in children with influenza or varicella infections. Fewer cases of Reye's Syndrome were reported this last winter probably due to the lower incidence of influenza infection. Improvements in the diagnosis and treatment of this disease have been made and mortality has been reduced to about 20%.

The portfolio of influenza research projects reflects the current period of fiscal restraint. Aggregate expenditures for influenza were reduced by about 4.5%; grant supported research grew by about 20% while contract supported research decreased by about 50%. The trend in grant supported research for influenza is encouraging but, the erosion of contract supported research is a serious concern. If continued, this erosion will result in the inability of the NIAID to exploit research developments arising from grant research which offer promise for the control of this important disease.

Viral Respiratory Diseases Program

The morbidity from illnesses due to parainfluenza viruses types 1, 2, and 3, and respiratory syncytial virus (RSV) is difficult to document, but it is estimated that 17 percent of pediatric infections are due to these agents. Of all severe lower respiratory diseases in infants and young children, perhaps 40 percent could be prevented if effective vaccine against these agents were available and used.

Elucidation of the role of fusion protein (F) in paramyxovirus spread in tissue culture systems has provided new impetus to identifying such a component in parainfluenza 3 and RSV. Studies are underway at the University of Alabama for identification, isolation and purification of surface glycoproteins of para-3 for potential use as vaccines.

Information is being generated on the biochemical and biophysical characteristics of RSV. Georgetown University has developed a series of monoclonal antibodies to a number of polypeptides of the virus, and is attempting to associate structure with function. Dr. Marcel Pons, an NIAID grantee, has characterized the genome as a single-stranded RNA, molecular weight of 5.0×10^6 daltons, and negative stranded. Infected cells contain 8-9 distinct messenger RNAs which is consistent with 8-9 identified viral polypeptides.

Progress on these human pathogens has been slow and difficult. With the development of basic information as to the structure and function of the viruses, coupled with knowledge of epidemiology and natural history of the diseases they cause, perhaps new and innovative approaches to prophylaxis and/or treatment can be formulated.

Viral Hepatitis Program

The goal of the Hepatitis Program is to develop control measures for hepatitis A (HAV), B (HBV), and non A-non B (NANB) infections. Because of its more serious clinical manifestations and long-term sequelae, HBV is of major public health importance in the U.S. It has been estimated by the Centers for Disease Control that 200,000 HBV infections occur each year in the U.S., with resultant costs of approximately $750,000,000. Of those infected, about 2.4 percent will die from cirrhosis and primary hepatocellular carcinoma, diseases closely associated with long-term chronic infection. Moreover, the global impact of the disease has even greater significance because it has been estimated that 176,000,000 chronic carriers serve as a reservoir of infection.

During the past year, data were accumulated on the reactogenicity and immunogenicity of the NIAID HBV vaccine in children, ages 1-17 years. The vaccine was non-reactogenic and highly immunogenic. Greater than 50% of all children developed anti-HBS within two weeks of the primary inoculation, and 78% of the one to two year olds developed anti-HBS within two weeks. Furthermore, 46/46 children, ages 1-10 years, developed antibody four weeks after the one-month booster. This same lot of vaccine is currently being used in the People's Republic of China for evaluation of vaccine in preventing maternal-fetal transmission of HBV. This trial is a randomized, placebo-controlled trial, and is being performed under a bilateral agreement between the U.S. (CDC and NIAID) and the PRC.

The code has just been broken for the efficacy study of HBV vaccine for "at-risk" hemodialysis patients and staff. The vaccine for this study was provided by Merck Sharpe & Dohme; their vaccine was approved for licensure during this past year. Early analysis of the data reveals that the vaccine is highly protective against infection in staff, confirming results from studies in homosexual populations. An extremely important aspect of the study was the demonstration of subtype cross-protection, e.g., the vaccine was composed of inactivated HBsAg adw but was efficacious against ayw virus. The efficacy data from the patient population are not definitive, and further analyses are required. This will be discussed in greater detail in the next report.

Evaluation of synthetic polypeptides (prepared by Dr. R. Lerner, Scripps Clinic) have continued during the past year. Antigen plus adjuvant have been administered to chimpanzees. Thus far, immunogenicity has been minimal. More recently, antigen coupled to tetanus toxoid has been used to boost the previously immunized chimpanzees. If stable antibody develops, the animals will be challenged with infectious virus to determine protective effects of the antibody.

Experiments are in progress to determine the effects of woodchuck hepatitis virus (WHV) vaccine on prevention of infection and impact on maternal-fetal transmission of virus. The vaccine (prepared by Dr. J. Gerin) consists of highly purified, formalin-inactivated WHsAg. The assays for WHsAg, anti-WHe, and DNA polymerase were developed by Georgetown University and the LID. A pool of infectious WHV has been titrated in woodchucks and has a titer of greater than or equal to 10^8 WID_{50}. Most of the pieces are now in place to perform appropriate experiments for characterizing the woodchuck as a model of human disease and its long term sequelae. The critical need is for an experimental facility for the studies. Without such a facility, work cannot continue.

Characterization of the delta agent continues at Georgetown University. The agent, a 37nm particle with HBsAg coat and RNA genome (M.W. of $5x10^5$ daltons), is directly pathogenic, and serial passage in HBV-carrier chimpanzees is associated with an increase in the severity of the disease. During the course of an outbreak of severe hepatitis B in Venezuela, there was a strong association between infection with delta agent and severe hepatitis.

As reported last year, the New Mexico State University chimpanzee colony (jointly supported by the NIAID, CDC, and NCDB) continues to serve as a valuable resource to the Hepatitis Program. Animals are brought to Meloy Laboratories where they are used for experiments designed by NIAID intramural investigators. The chimpanzees are cycled through studies with respiratory syncytial virus, hepatitis A virus, hepatitis B virus, and NANB viruses.

Antiviral Substances Program

The need for safe, effective chemoprophylactic and therapeutic agents is well recognized. While certain viral diseases are effectively controlled by vaccines, others are not amenable to this approach. One of the major difficulties in the development of successful antiviral agents is the host cell toxicity that so frequently is associated with the antiviral effect. What is needed is to define some unique step in the viral infectious process that can be inhibited without significantly affecting the metabolism of uninfected cells. This requires an in-depth understanding of mechanisms of virus replication in the search for potential targets. The field is still in its infancy. Most available drugs have been discovered by accident. Compounds such as acyclovir and bromovinyldeoxyuridine, however, offer some promise since they rely on a virus-induced enzyme, HSV thymidine kinase, for activation. This explains why their therapeutic index is relatively high compared to other available drugs. This issue of drug toxicity also makes it clear why interferon, a natural antiviral substance, is so attractive an agent.

The ASP supports a broad spectrum of research that ranges from basic research on the mechanism of action of antivirals and interferon through large, controlled efficacy studies. Promising antiviral agents can be evaluated in a variety of animal model systems for both efficacy and toxicity. If these preclinical studies indicate potential for use in man, clinical trials will be initiated in those cases where federally sponsored studies are appropriate. The Program is careful not to duplicate or supplant the role of industry in such efforts, but often works cooperatively to obtain data on promising agents, such as bromovinyldeoxyuridine.

This year saw the addition of a new compound to the small but growing list of FDA approved antiviral drugs. Acyclovir (Zovirax) is a synthetic purine nucleoside analogue developed by the Burroughs Wellcome Company which shows activity against several members of the Herpesvirus group. The approved indications for acyclovir at this time are limited to primary genital herpesvirus infections and limited nonlife-threatening mucocutaneous herpes simplex virus infections in immunocompromised individuals. Clinical studies supported by the DAB were unable to demonstrate a significant effect of topical acyclovir treatment on clinical disease in normal individuals suffering from herpes labialis. Therefore, although there is modest benefit associated with topical acyclovir treatment in some limited situations, more effective formulations are necessary.

Analysis was completed this year of a trial of vidarabine (adenine arabinoside) for treatment of varicella-zoster virus infections in the immunocompromised host. The results were published in the New England Journal of Medicine and showed that early treatment accelerated cutaneous healing and decreased both cutaneous dissemination, visceral complications and the duration of post-herpetic neuralgia. Licensure of vidarabine for all zoster infections in the immunocompromised host, predicated upon these data, is imminent. The results of a trial of vidarabine for treatment of chicken pox in immunocompromised patients were also published this year in the Journal of Pediatrics. These findings indicated that vidarabine has a good therapeutic index when given early in the course of infection.

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While HSV infections can be quite severe in the immunosuppressed individual, one should not underestimate the physical and psychosocial trauma of genital HSV in the normal host. Recent data have confirmed a dramatic increase in the incidence of this disease. A controlled trial of parenterally administered interferon in females with initial genital infections is currently being conducted. It will evaluate the effect on both the initial infection and the frequency and severity of recurrences. Since this study is blinded, no results of treatment are yet available.

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Bacterial Vaccines Program

Pneumococcal Diseases: Since the late 1960's, the NIAID has sponsored studies on the development and clinical testing of pneumococcal polysaccharide vaccines. Data from these studies along with data generated by manufacturers led to the licensure of a polyvalent pneumococcal vaccine in 1977. Since licensure, the NIAID has collaborated with a number of investigators studying the vaccine in patients with conditions predisposing to pneumococcal infections. Although none of these studies are directly supported by NIAID, they are coordinated by the Bacterial Vaccines Program Officer, and all antibody assays were done by the contract-supported reference laboratory at SUNY, Downstate. Patients with the following conditions appear to mount normal antibody responses to pneumococcal vaccine: juvenile and adult insulin-dependent diabetes, nephrotic syndrome (steroid sensitive), chronic obstructive pulmonary disease, systemic lupus erythematosus (SLE), renal transplant, Hodgkin's disease (before chemo- and radiotherapy), and sickle cell anemia. Patients with SLE but on therapy, hemodialysis patients, renal transplant patients less than six months post-operative, and splenectomy patients seem to mount a more moderate response. Several types of patients, however, respond poorly to pneumococcal vaccine: those with nephrotic syndrome (steroid resistant), chronic renal failure, Hodgkin's disease on immunosuppressive treatment, and multiple myeloma. It is apparent that immunosuppression, either via treatment or as a consequence of disease, is more important in determining response to the vaccine than the condition itself.

Otitis media is one of the most frequent infections of the infant and young child. Fluid may persist in the ears of children for weeks or months following acute infection and may cause hearing disorders with subsequent learning disabilities. Because of the NIAID's involvement with pneumococcal vaccine and because pneumococci are a major cause of acute otitis media, a program to study the value of pneumococcal vaccine in preventing otitis media was started in 1975. Efficacy trials were performed at Boston City Hospital and Huntsville (Alabama) Hospital and antibody response studies in infants were done at Vanderbilt. Efficacy trials, not NIAID supported, were also done in Finland. Data from all these studies lead to the following conclusions: (1) the infant serum antibody response varies with the different polysaccharides; some polysaccharides evoke a good response while others give a poor or no response, (2) the serotypes causing most of the otitis media are those whose polysaccharides are poor immunogens in infants, (3) the vaccine does show type specific protection against types which are good immunogens; unfortunately, as those types account for only a small portion of the disease, the present vaccine has little effect on the overall otitis media. It is apparent that the production of an effective otitis media vaccine must await the development of polysaccharides which are immunogenic in infants.

Meningitis: It is estimated that approximately 20,000 cases of bacterial meningitis occur in the U. S. each year, mostly in young children and infants. For much of the past decade, program efforts have been directed toward development of vaccines for the protection of children under two years of age in whom most of this disease occurs. A purified capsular polysaccharide (polyribose phosphate, PRP) vaccine of *Haemophilus influenzae*, developed with NIAID support, has been shown to be non-immunogenic and ineffective in infants. A more recent candidate vaccine containing cell envelope proteins complexed with PR has been the disappointing subject of clinical trials and has essentially been abandoned. Several contracts and grants within this program are directed at exploring new approaches to the development of a vaccine for use in infants. The approaches include: (1) extraction and characterization of outer membrane proteins as potential antigens, (2) preparation of complexes of pure PRP with various pure proteins such as diphtheria toxoid, (3) preparation of molecules of PRP containing increased numbers of carboxyl groups to increase immunogenicity, and (4) use of cross-reacting polysaccharides such as those from *E. coli* K-100.

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The Development and Applications Branch also supports five General Vaccine Evaluation Units that form the backbone of the Branch's efforts for approaches to the control of important disease problems. Each unit has specialized population groups available to them, i.e., normal adults, pediatric groups, "at-risk" adults and/or children, for evaluating viral vaccines, bacterial vaccines, antiviral drugs, and environmental controls. Epidemiologic studies performed by several of the units have provided much of our current knowledge on the natural history of several of the respiratory agents. During the past year the evaluation units have tested candidate vaccines such as attenuated influenza vaccines in children and adults, hepatitis B vaccines in children, *H. influenzae* and meningococcal vaccines in adults and children; they have also tested antivirals such as amantadine, rimantadine and interferon.

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The Viral Vaccine Program covers those areas where research should be stimulated toward the goal of an effective vaccine, but are not covered by the other specific programs. As previously reported, New York University is evaluating an attenuated varicella vaccine, under contract, in immunosuppressed children. Humoral and cellular immune responses in fifteen normal adults have been analyzed. To date over 100 children and approximately 20 adults have been vaccinated; about 10 of these individuals have had close exposures to infected cases. The only possible side effect has been that a few children with chemotherapy suspended developed maculopapular lesions about one to two weeks after vaccine. V-Z virus was not isolated from them, nor has virus shedding been documented in any vaccinee. There has been no indication of vaccine virus spread. All vaccinees tested have responded immunologically.

The Development and Applications Branch also supports five General Vaccine Evaluation Units that form the backbone of the Branch's efforts for approaches to the control of important disease problems. Each unit has specialized population groups available to them, i.e., normal adults, pediatric groups, "at-risk" adults and/or children, for evaluating viral vaccines, bacterial vaccines, antiviral drugs, and environmental controls. Epidemiologic studies performed by several of the units have provided much of our current knowledge on the natural history of several of the respiratory agents. During the past year the evaluation units have tested candidate vaccines such as attenuated influenza vaccines in children and adults, hepatitis B vaccines in children, *H. influenzae* and meningococcal vaccines in adults and children; they have also tested antivirals such as amantadine, rimantadine and interferon.

MOLECULAR MICROBIOLOGY AND PARASITOLOGY BRANCH

The Molecular Microbiology and Parasitology Branch plans and conducts research grant, program project grant, contract, training grant, fellowship and career award programs in molecular microbiology (biochemistry, genetics, DNA recombinants, physiology), and parasitology and medical entomology. It also coordinates the activities of the Parasitic Diseases Panel of the U.S.-Japan Cooperative Medical Science Program.

Approximate Level of Support

Molecular Microbiology Section

Activity	Number	Amount
Research Grants 1/	212	$17,575,911
Training Grants	3	220,173
Fellowships	30	475,683
Subtotal	245	$18,271,767

1/ Includes 13 Career Awards ($503,866)

Parasitology Section

Activity	Number	Amount
Research Grants 2/	197	$15,478,493
Program Project Grants	2	755,307
Research Contracts	2	140,056
Training Grants	8	637,983
Fellowships	10	183,276
Subtotal	219	$17,195,115

2/ Includes 5 Career Awards ($158,236)

Branch

Activity	Number	Amount
Research Grants 3/	409	$33,054,404
Program Project Grants	2	755,307
Research Contracts	2	140,056
Training Grants	11	858,156
Fellowships	40	658,959
TOTAL	464	$35,466,882

3/ Includes 18 Career Awards ($662,102)

STAFF ACTIVITIES

A paper on "Research in Parasitology from an NIH Perspective" given by Dr. Delappe during the Rockefeller/Macy Conference in New Orleans, La. (Oct. 20-22, 1980) has been published in a book "The Current Status and Future of Parasitology."

A poster presentation given at the International Conference on Molecular Biology, Pathogenicity and Ecology of Bacterial Plasmids (Jan. 5-9, 1981-Santo Domingo, Dominican Republic) has been published by Plenum Press.

Dr. Delappe was invited to give one of five special lectures during The Third Tokyo Symposium on Microbial Drug Resistance held in Tokyo, Japan (Oct. 23-25, 1981). The title of the presentation was "Evolution of Support for Plasmid Research by the National Institute of Allergy and Infectious Diseases." This will be published in the near future.

During the period Nov. 9-12, 1981, Dr. Delappe attended the American Society of Microbiology International Conference on Streptococcal Genetics in Sarasota, Florida. A full report was submitted subsequently.

The 30th Annual Meeting (Nov. 16-20, 1981-San Juan, Puerto Rico) of the American Society of Tropical Medicine and Hygiene was attended by Drs. Sheffield and Delappe.

Dr. Sheffield will represent the Institute during the U.S.-Japan Cooperative Medical Science Program, Parasitic Diseases Panel in Kumamoto, Japan, Aug. 22-25, 1982.

Dr. Sheffield will attend the 5th International Congress of Parasitology in Toronto, Ontario, Aug. 8-14, 1982.

MOLECULAR MICROBIOLOGY

Program Summary

In addition to the basic free-ranging research of relevance to the Institute, there are two structured programs supported by this section of the Branch, one of which involves mechanisms of resistance to antimicrobial agents and the other, recombinant DNA.

(1) Mechanisms of Resistance to Antimicrobial Agents

During the past twelve years, the problem of microbial resistance to therapeutic agents has become increasingly apparent. The widespread and indiscriminate use of antibiotics with associated increase in resistance of gram-negative and other organisms make the management of hospital-associated infections increasingly difficult. Of growing concern is the increase in penicillinase-producing gonococci.

There is substantial clinical and epidemiological evidence that development of antibiotic resistance, and especially plasmid (extrachromosomal) mediated drug resistance, represents a growing problem in medical care. Most of the projects are concerned with defining the fundamental biological mechanisms involved in the development of drug resistance by microorganisms. Specific goals involve investigation of the origin, development, evolution, expression and mechanism of drug resistance in a variety of specific microorganisms. Examples of microorganisms of particular interest are (but not limited to): Haemophilus influenzae, Enterobacteriaceae, Pseudomonas, Neisseria, staphylococci, streptococci, mycobacteria, mycoplasmas, and pathogenic fungi.

Research Highlights

AI 15619-04A1 V. Burdett (Duke University): Certain characteristics of plasmids found in the Enterobacteriaciae are shared by streptococcal plasmids. But despite a considerable amount of information on drug-resistance factors in enterobacteria and interactions of these plasmids, the literature on drug-resistance plasmids in streptococci is still scarce. This laboratory is interested in the ecology and dissemination of resistance genes among β-hemolytic streptococci. As a preliminary step, they have developed a plasmid transfer system in group B streptococcus as a means for further study of plasmids in streptococci. Evidence is presented that R-plasmids (with molecular weights of $17\text{-}20\times10^6$) mediating erythromycin resistance in group B and D streptococci are capable of mediating their own transfer. Further, all erythromycin resistance plasmids from streptococci so far examined appear to constitute a family of DNA molecules with similar patterns of cleavage by restriction endonucleases.

AI 14107-06 R. K. Holmes (Uniformed Services University of the Health Sciences): One of Dr. Holmes' objectives is to analyze ferric iron transport in *Corynebacterium diphtheriae* and its relationship to control of toxigenesis. In wild type *C. diphtheriae*, production of diphtherial toxin is suppressed when high concentrations of iron are present in the growth medium. He previously isolated mutants of *C. diphtheriae* C7(B) that can produce large amounts of diphtherial toxin under high-iron conditions. Preliminary studies indicated that these mutants were deficient in their ability to transport iron. He has initiated a study of iron transport as a first step toward defining the relationships between iron transport and toxinogenesis in *C. diphtheriae*. He has demonstrated that iron transport into *C. diphtheriae* is specific for ferric iron and occurs by active transport. Maximal rates of Fe^{3+} uptake were observed at pH 7 and 37°C using cells grown under iron limiting conditions in the presence of a metabolizable carbohydrate source. This system does not catalyze the exchange of extracellular iron for cell-associated iron. The nature of the energy requirement for transport was investigated by using a variety of metabolic inhibitors, uncouplers and ionophores. Concentrations of these compounds required to effect Fe^{3+} transport were significantly higher than those reported for other bacteria. His data are most consistent with the hypothesis that the electrochemical gradient serves as the major source for ferric iron transport in *C. diphtheriae*.

AI 16806-02 B. Cooperman (University of Pennsylvania): A group of 21 tetracyclines have been ordered with respect to their growth inhibitory activity for a tetracycline sensitive strain of *E. coli* and one of *S. aureus*. With but a few exceptions, the same relative order of activity holds for the inhibition of polyphenylalanine synthesis with cell-free extracts of these strains, and for the inhibition of phenylalanyl-tRNA binding to the ribosomes of the *E. coli* strain. Polyphenylalanine synthesis in *S. aureus* extracts is 6.6-fold more sensitive to tetracycline inhibition than the corresponding synthesis in *E. coli* extracts, in agreement with the differential susceptibility of these two strains determined from steady state growth kinetics. The results tend to indicate that binding of the tetracycline antibiotics at their ribosomal target site is the rate limiting event in the susceptibility of sensitive bacteria.

AI 16756-02 S. Levy (Tufts University): The overall objective of this research is to describe and determine the mechanism for resistance to tetracycline and its analogs. Lipophilic tetracycline analogs have been developed which have activity against tetracycline-resistant coliforms. However, Dr. Levy has recently shown that over 70% of naturally occurring tetracycline-resistant *E. coli* are already resistant to clinically useful levels of the analogs, e.g. minocycline. This common analog resistance determinant is encoded on the genetic element, transposon 10. Other tetracycline analogs which have been developed have been tested in the system with different resistance plasmids. In general, these drugs are all more lipophilic than tetracycline. One such drug, thiatetracycline, is the most exciting since it has proven to be effective against tetracycline resistant organisms, including those encoding resistance to the analogs. In order to understand the pharmaco-physiologic effects of the analogs, he has been studying their uptake and efflux in sensitive and tetracycline-resistant cells. By studying the differences between two tetracyclines, tetracycline and minocycline, he hopes to decipher the mechanism for their relative effectiveness. Dr. Levy has determined from the minimal inhibitory concentrations (MIC) in liquid culture that minocycline is relatively more effective against *E. coli* resistant cells than against sensitive cells. Kinetic data using labeled drugs have confirmed this finding. Minocycline is actively transported at a rate 10 times faster than tetracycline, but is effluxed only .25 times the rate of tetracycline. Consequently, minocycline is able to achieve internal inhibitory concentrations at much lower external levels than tetracycline. Dr. Levy believes that the resistance phenotype is chromosomally determined since efforts to locate plasmid DNA have been unsuccessful. These have included multiple physical plasmid screening techniques and filter conjugal matings.

AI 10492-11 N. Groman (University of Washington): The overall objective of this project is to study the genetic relationship of Corynebacterium diphtheriae and other aerobic corynebacteria, and the genetic exchange mechanisms which may influence those relationships. In addition to bacteriophage, one of the means by which genetic material could be transmitted among the corynebacteria is by plasmids. Dr. Groman recently reported the first isolation of plasmids from C. diphtheria and associated skin diphtheroids. His group has now examined a number of skin diphtheroids for plasmid carriage. Of the 46 strains examined, 12 (26%) contain one or more plasmids. It is his intent to examine the relationship of some of these plasmids to each other and to the one plasmid found thus far in C. diphtheriae. The possibility that plasmids are exchanged between skin diphtheroids and between them and C. diphtheriae needs to be examined. Efforts to demonstrate plasmid transmissibility have not been successful as yet. It is possible that, as in Staphylococcus aureus, such exchanges are mediated by bacteriophages.

AI 13948-06 J. Ensign (University of Wisconsin): Dr. Ensign has explored the activity of a germination inhibitor released during germination of Streptomyces viridochromogenes. This inhibitor, also an antibiotic against some gram positive bacteria, was shown to be an inhibitor of membrane-bound ATPase activity. This is the first documentation of a specific inhibitor involved in dormancy and germination of a bacterial spore system. The inhibitor has been characterized with respect to general properties and size but its structure has not been determined. He found that addition of calcium ions to heat- or detergent-activated spores was sufficient to initiate germination. This initiation of germination begins with release from the target site of the germination inhibitor resulting in coupling of transport and production of ATP. A key to understanding the action of the inhibitor in establishing and maintaining dormancy is the mechanism of its release. Attempts to release it from membrane preparations prepared from dormant spores have not yet been successful.

(2) Recombinant DNA Molecular Research

During the past few years, the development of certain techniques in the area of molecular biology has made it possible to construct functional DNA molecules in vitro which contain segments derived from diverse biological sources. The scientific innovations which led to this technological breakthrough were mostly derivative from basic studies on the mechanism of restriction, which normally acts as a barrier to gene flow among microorganisms, and on the molecular biology and genetics of bacterial plasmids, especially those specifying drug resistance. Grantees of this Institute played a preponderant role in both of these areas. Whereas DNA recombination in nature has depended on random processes, the experimental techniques now available enable the in vitro construction and subsequent replication of DNA molecules needed for specific experimental goals. This basic advance, coupled with refinements of the existing technology, should provide greater knowledge of the mechanisms of pathogenicity (at the molecular level) of viral, bacterial, mycotic and parasitic agents. This information, in turn, may lead to improved prevention, diagnosis and treatment of infectious diseases. Notwithstanding the potential benefits of recombinant DNA molecule research, there may be associated potential biohazards which must be avoided by the design, construction and testing of safer host-vector systems for use in these studies.

The primary goal of this research is the development and utilization of recombinant DNA molecule technology to increase fundamental knowledge and ultimately enhance control of the etiological agents of infectious diseases. Another goal is the synthesis of a variety of biologically useful substances through the construction of bacterial cells containing functional DNA of either plant or animal origin. An equally important goal is the identification, assessment, and elimination of any and all potential biohazards encountered in the exploitation of this technology.

Research Highlights

AI 10106-12 J. Konisky (University of Illinois): Dr. Konisky continues his studies of the mode of action of I-colicins on *Escherichia coli*. Addition of purified colicin Ib to whole *Escherichia coli* cells or cytoplasmic membrane vesicles inhibits their subsequent ability to generate a membrane potential. In addition, this colicin is shown to bring about a voltage-dependent increase in the conductance of an artificial planar bilayer membrane prepared from soybean phospholipids. This results from the formation of ion-permeable channels. These data provide strong evidence that the depolarization of *Escherichia coli* cells by this colicin results from an Ib-induced increase in membrane permeability to ions.

The colicin V immunity and structural genes of plasmid pColV-B188 have been cloned into the vectors pMB9, pBR322 and pMK16. Both genes are closely linked and can be isolated on a 900 base pair DNA fragment. Insertion of the transposon Tn5 into this cloned sequence led to the construction of a mutant plasmid which conferred colicin V immunity, but not ability to produce this colicin. Analysis of the products determined by these cloned genes in maxicells has led to the conclusion that the polypeptide involved in immunity has a molecular weight of about 6,500, while the colicin has a molecular weight of approximately 4,000.

AI 8619-15 S. Cohen (Stanford University): Dr. Stanley Cohen and coworkers continue their research on cloning and expression in *Streptomyces lividans* of antibiotic resistance genes derived from *Escherichia coli*. *Streptomyces* spp. are gram-positive, mycelial organisms that undergo a complex developmental life cycle and produce a variety of medically important antibiotics as secondary metabolites. Recently, the establishment of an efficient transformation system for *Streptomyces* spp. and the development of endogenous plasmids and phages as cloning vehicles have provided the tools for investigating the molecular genetics of these organisms. Hybrid plasmids able to replicate in both *Escherichia coli* and *Streptomyces* species, and containing selectable genes capable of phenotypic expression in each of the hosts, could greatly facilitate the structural analysis of *Streptomyces* genes and the study of factors that regulate the expression of genetic information transplanted between these hosts. During investigations intended to identify DNA sequences that accomplish transcriptional and translational control in *Streptomyces* spp. it was observed that two antibiotic resistance genes derived from *E. coli* were expressed in *S. lividans*. It is now possible to use DNA cloning to examine the organization and regulation of antibiotic production pathways in *Streptomyces* spp. in an effort to produce new and possibly more effective antibiotics.

(3) Other Basic Research

AI 06553-01 J. Spanier (Cornell University): Dr. Spanier has studied heavy metal and antibiotic resistance in the bacterial flora of sediments from the New York Bight. The New York Bight extends seaward some 80 to 100 miles from the Long Island and New Jersey shorelines to the edge of the continental shelf. Over 14 X

$10^6 m^3$ of sewage sludge, dredge spoils, acid wastes, and cellar dirt are discharged into this area each year. Large populations of Bacillus sp. resistant to 20 mg of mercury per ml were observed in Bight sediments contaminated by these wastes. Resistant Bacillus populations were much greater in sediments containing high concentrations of mercury and other heavy metals than in sediments from areas further offshore where dumping has never been practiced and where heavy metal concentrations were found to be low. Ampicillin resistance due mainly to β-lactamase production was significantly ($P < 0.001$) more frequent in Bacillus strains from sediments near the sewage sludge dump site than in similar Bacillus populations from control sediments. Bacillus strains with combined ampicillin and Hg resistances were almost six times as frequent at the sludge dump site as in control sediments. This observation suggests that genes for Hg resistance and β-lactamase production are simultaneously selected for in Bacillus and that heavy metal contamination of an ecosystem can result in a selection pressure for antibiotic resistance in bacteria in that system. Also, Hg resistance was frequently linked with other heavy metal resistances and, in a substantial proportion of Bacillus strains, involved reduction to volatile metallic mercury.

AI 04769-20 K. Rinehart (University of Illinois): The past year has provided exciting results. Structures have been assigned to didemnins A, B, and C, isolated from a Caribbean tunicate (a Trididemnum species). Didemnin B, the most active, has been shown to extend the lifetime of mice infected with P388 leukemia (T/C 199) and B16 melanoma (T/C 160) and 15 grams of the compound is being isolated (under a separate contract with the National Cancer Institute) for clinical testing. Didemnin B has also been shown to be effective in treating vaginal Herpes simplex II infections in mice, and has recently been shown to be effective in treating Rift Valley fever virus in mice. Most recently, Dr. Rinehart discovered several new didemnins and these are undergoing biological testing.

In the marine natural products area he has also completed assignments of structures to ptilocaulin and isoptilocaulin, cyclic guanidines found in three marine sponges which were identified in the 1978 Alpha Helix Caribbean Expedition as being among the most intensely antibacterial, antifungal and cytotoxic species investigated. Finally, he has completed the structural assignment of pyochelin, a growth promoter for Pseudomonas aeruginosa, including strains isolated from clinical burn patients. In the coming year, Dr. Rinehart hopes to identify new didemnins and provide samples for in vivo testing against Lhasa fever and other RNA viruses as well as Herpes simplex and other DNA viruses.

PARASITOLOGY

Program Summary

In addition to the basic free-ranging research of relevance to the Institute, there are two structured programs supported by this section of the Branch, biological regulation of vectors and immunology of parasitic infections.

(1) Biological Regulation of Vectors

This program has as its goal the advancement of fundamental studies which might lead to effective methods of biological regulation of vectors. For the past 30 years, control of pests and disease vectors has been based primarily on the use of synthetic organic compounds which had the "advantages" of long residual action and toxicity to a broad spectrum of target organisms. It has now been shown that, because of these very characteristics, many of these pesticides are more deleterious than beneficial when all effects on man and his environment are considered. Furthermore, resistance to broad spectrum chemical pesticides has reduced their effectiveness in many vector control programs. For these reasons the search for alternative methods of pest control has become imperative, and it is generally agreed that the best approaches will consist of integrated pest management programs which combine biological control, in the broadest sense, with the judicious use of more specific chemicals and management of the physical environment. This approach to vector control must be based on adequate information about the ecology of the target organisms in which the control program is to be conducted, effects of control measures on nontarget organisms in the environment, and the biology of the disease organism being transmitted.

Research Highlights

AI 10987-09 D. Sonenshine (Old Dominion University): Studies on the sex pheromone, 2,6-dichlorophenol, in ticks has shown that the compound is present in neutral lipid droplets, composed mostly of triacylglycerides and cholesterol esters, in the foveal glands of the tick. The composition of the foveal gland fatty acids suggests a storage and/or secretory function. Use of lipid solutions for storage masks the toxicity of the sex pheromone and facilitates its transport to the surface of the tick. Incorporation of synthesized sex pheromone in lipid droplets may provide an effective means of dispersal of the attractant in vector control measures.

AI 17711-03 J. Hildebrand (Harvard University): Ultrastructural study of the antennal lobe of the brain of *Manduca sexta* indicates that the structure is complex in both morphology and physiology and functions to filter or integrate olfactory information before transmission to the protocerebral centers of the insect. The lobe comprises a central area of coarse neuropil surrounded by dense spheroidal glomeruli where all synapses between antennal-nerve axons and second order neurons of the lobe occur. A macroglomerular complex found in the antennal lobes of the male, but not the female, processes inputs from receptor neurons specialized to detect sex pheromone and is intimately involved in the mating process.

AI 09503-13 W. Harvey (Temple University): In basic studies on the intestinal epithelium during maturation in insects, a technique has been developed for isolation, in highly purified form, of the apical plasma membrane of the goblet cells. Particles, termed portasomes and found on the goblet cell apical membrane, are similar to particles of the F_1-F_o ATPase of phosphorylating membranes. The portasomes are involved in K^+ transport across the gut epithelium much like the F_1-F_o particles are involved in H^+ transport. Further studies using this technique are in progress to elucidate the action of *Bacillus thuringiensis* toxin which is a potent inhibitor of the K^+ pump and a potential insecticide.

(2) Immunology of Parasitic Infections

The complexity of structure and function of parasites has made the study of the immunology of these infectious agents exceptionally challenging and rewarding. Exciting opportunities for the elucidation of mechanism and manifestations of immunological responses to parasites now exist as the result of the impressive developments in immunology in recent years. Major ultimate goals of studies on the immunology of parasitic infections are the development of effective vaccines for the prevention of parasitic diseases (such as malaria, schistosomiasis, and filariasis), the intervention in the host response to prevent or ameliorate disease processes which are immunologically mediated, and the development or improvement of immunodiagnostic procedures for parasitic infections, especially as they relate to the immune status of the host.

A related goal of these studies is to contribute to an understanding and solution of basic and clinical problems associated with other disease entities, especially immunological disorders and hypersensitivity states. A number of parasitic infections are excellent models for such studies as (a) the mechanisms of intracellular immunity, (b) the enhancement of suppression of concurrent infections or tumor development, (c) immunopathological mechanisms, (d) development of disease process in immunosuppressed or immunostimulated hosts, (e) the biochemical and genetic mechanisms for the development of pathogen variants with different immunological characteristics, (f) the genetic basis for variations in host response, and (g) the role of IgE and other cytotropic antibodies in hypersensitivity.

Research Highlights

AI 16740-02 R. Bell (Cornell University): In studies on the genetics of resistance to *Trichinella spiralis*, the responsiveness of various inbred mouse strains following phase-specific immunization with *T. spiralis* has suggested that the major genes controlling anti-preadult and anti-adult responses are not linked to the major histocompatibility complex (MHC) although several minor genes linked to it exert a degree of control over responsiveness. As a result of these studies, the effects of two separate sets of genes have been dissociated relative to their linkage to the MHC. Rapid expulsion of worms has been functionally defined and evidence has been provided for the first time of a single dominant autosomal gene controlling the rapid expulsion phenomenon.

AI 13802-06 M. Dresden (Baylor College of Medicine): Using a newly developed procedure for isolation of schistosome eggs, sufficient egg protease has been obtained for purification and characterization studies. One of five monoclonal antibodies derived from infected mice has shown high inhibitory activity against the egg protease. This monoclonal antibody will aid in localization of the protease in the schistosome egg and in its purification. Further studies should yield specific information on the biochemistry of penetration of host tissues. Isolation of the lytic enzymes elaborated by the egg may aid in the identification of a major antigen which is instrumental in the inflammatory process around the egg and in other immunopathological responses in schistosomiasis.

AI 16639-02 A. Vincent (University of South Florida): In the study of rodent filarial models, it has been shown that in the nude mouse large intraperitoneal inoculations of L_3 larvae of *Brugia pahangi* result in high yields of microfilaria and adult worms which are a good source of antigen. At three months post-inoculation yields of 2×10^6 microfilariae were obtained and approximately one fourth of the inoculum was recovered as adult worms. Examination of the developmental and growth characteristics for *B. pahangi* in the mouse model indicated that growth, development and molting closely paralleled *B. pahangi* infections in cats. However, attempts to induce bancroftian filariasis were unsuccessful. Using the nude mouse model this investigator has shown that significant lymphatic changes such as fibrosis and dilation appear to be T-cell independent whereas others like severe necrotizing vasculitis and granuloma formation are T-cell dependent. The immune basis of filarial lymphatic lesions remains an unanswered question but one which this model is uniquely qualififed to address.

AI 15351-04 A. Mahmoud/J. Ellner (Case Western Reserve University): In man infected with *Schistosoma mansoni*, the correlation among degree of exposure, intensity of infection and development of hepatosplenomegaly is inexact. This suggests that variability in the host immune response modifies the expression of infection and disease. In age-matched groups of patients with *S. mansoni* these workers demonstrated that ^3H thymidine incorporation in peripheral blood mononuclear cells induced by the non-specific mitogen, phytohemagglutinin (PHA) and the antigens, streptokinase-streptodornase (SKSD) and schistosome soluble egg antigen (SEA), did not differ between patient groups with light or heavy infections. However, response to a soluble worm antigen preparation (SWAP) was inversely proportional to the intensity of infection. In 20 hepatosplenomegaly patients, 7 were unresponsive to SWAP and 13 were highly responsive. The former had comparable PHA and depressed SKSD and SEA reactivity as compared to the SWAP high responders. This is the first demonstration of dichotomies in the immune response of S. mansoni infection which may influence expression of infection and contribute to the pathogenesis of the disease.

AI 16479-03 J. David (Harvard Medical School): Circulating eosinophils isolated from the blood of eosinophilic patients have the metabolic and surface charge characteristics of activated cells and have an enhanced helminthotoxicity in vitro. Such cells damage antibody- or complement-coated schistosomula when incubated in medium containing eosinophil colony stimulating factor (EO-CSF). In contrast to normal eosinophils, the activated eosinophils adhere to Con-A coated schistosomula and some adhere to uncoated worms. Purified EO-CSF does not modify eosinophil adherence at 4°C that is mediated by Fc receptors in absence of degranulation. These results suggest that EO-CSF enhances helminthotoxicity by affecting the degranulation step of the killing reaction. The finding that patients with schistosomiasis do not produce this enhancing factor may explain why their eosinophils do not eliminate the schistosome larvae in vivo.

AI 17429-02 R. Nussenzweig (New York University): In studies on protective antigens of Plasmodium falciparum sporozoites a monoclonal antibody has been produced which is active against the sporozoite. An isolate from the parasite, having a mol. wt. of approximately 72,000, was precipitated both by the monoclonal antibody and by serum from a volunteer immunized with a geographically distinct isolate of P. falciparum indicating that the strain used to produce the hybridoma shared a common surface antigen with that from the volunteer. The isolated protein was species specific. Recent experiments with two chimpanzees showed that protection occurred in one of the two when sporozoites were incubated in the Fab fragments of the monoclonal antibody before inoculation. Since a protective antigen has been demonstrated in rodent malaria using this technique, it is anticipated that one can be isolated for the human malarial parasite which would be a significant advance in the development of a vaccine.

AI 14848-03 F. Kierszenbaum (Michigan State University): Eosinophils participate in antibody-dependent cell mediated cytotoxicity (ADCC) reactions attacking a variety of parasites. In some damage is associated with degranulation of the eosinophils with release of major basic protein (MBP) onto the parasite surface. Heparin inhibition of eosinophil-effected ADCC was tested to determine whether MBP promotes adherence and killing of bloodstream forms of Trypanosoma cruzi. The demonstration of a concentration-related inhibition supports a previous finding that MBP has a direct toxic effect on T. cruzi and suggests that MBP and/or other basic molecules are the mediator released by eosinophils causing adherence or lysis of the trypanosome.

AI 17913-03 R. Leid (Washington State University): The larval stage of the tapeworm, Taenia taeniaeformis, is a tissue-dweller and is able to evade the host's defense mechanisms. During studies of the modulation of inflammation, Dr. Leid has demonstrated a parasite-derived protease inhibitor of inflammation. This inhibitor is active against trypsin-like enzymes of the human complement cascade and also reacts with other plasma enzymes. He also has found that arachidonic acid is incorporated by tapeworms with production of thromboxane A(TXA_2), a potent platelet antagonist. The latter finding opens a whole new area of research on parasite modulation of host systems.

(3) General Parasitology Research Highlights

AI 14993-05 J. Bennett (Michigan State University): With the goal of developing more effective and more selective drugs for treatment of schistosomiasis, studies have concentrated on the physiology of nerve and muscle of the parasite. Electrical characteristics of the muscle and the tegument indicate an intimate association between the two with the possible implication that a drug having major activity against muscle may exert its action indirectly by affecting tegument. However, experimental removal of the tegument has little effect on muscular function although the tegument has a role in regulation of the ionic milieu for the muscle cells. Electrical potential studies of whole worms have promise as a means of evaluating drug activity (sensitivity is several hundred fold greater than standard techniques) but excessive "noise" is a problem to be solved.

AI 15070-03 G. Hill (Colorado State University): Cultivation of infective trypomastigotes of *Trypanosoma brucei rhodesiense* has been achieved using 10% fetal bovine serum. Studies on the electron transport system have shown that the predominant terminal oxidase in these organisms is these organisms is the salicylhydroxamic-sensitive L-alpha-glycerol phosphate oxidase system. The cultured organisms remain highly infective to mice and rats. Organisms also can undergo transformation from infective to procyclic trypanosomes, an important part of the life cycle, in culture. The rate of transformation is markedly increased in the presence of cis-aconitate and sodium pyruvate.

(4) Contract Activity

The schistosomiasis supply contract at the University of Lowell has continued to provide all three human schistosome species and their vector snails to many schistosomiasis researchers in this country.

The filariasis supply contract at the University of Georgia which provides research workers with filariae and the appropriate vectors has greatly contributed to the acceleration of research on filariasis.

EXTRAMURAL ACTIVITIES PROGRAM

NIAID

TABLE OF CONTENTS

REPORT OF THE DIRECTOR.......... 11-1

- A. Introduction.......... 11-1
- B. Personnel.......... 11-2
- C. Committee Management.......... 11-3
- D. Conference Proposals.......... 11-3
- E. Transfer of Research Grant Applications to Others BIDs.......... 11-4
- F. Study of Priority Scores and Study Section Behavior.......... 11-5

I. CONTRACT MANAGEMENT BRANCH.......... 12-1

II. GRANTS MANAGEMENT BRANCH.......... 13-1

III. PROGRAM AND PROJECT REVIEW BRANCH.......... 14-1

- A. Allergy, Immunology and Transplantation Research Committee, NIAID.......... 14-1
- B. Allergy and Clinical Immunology Subcommittee.......... 14-1
- C. Transplantation Biology and Immunology Subcommittee.......... 14-1
- D. Microbiology and Infectious Diseases Advisory Committee.......... 14-3
- E. Review Services Unit.......... 14-5

IV. RESEARCH RESOURCES SECTION.......... 15-1

- A. Special Activities.......... 15-1
- B. Allergen Reagents.......... 15-1
- C. Immunological Reagents and Resources.......... 15-2
- D. Microbiological Reagents.......... 15-2
- E. Research Resources - Research Reagents.......... 15-3
- F. Processing and Distribution.......... 15-3

REPORT OF THE ACTING DIRECTOR, EAP

A. Introduction

FY 1982 saw a certain type of activity in EAP not previously experienced in the 1970's, primarily because of considerable uncertainties in the budget. In anticipation of the worst possible budget, all active grant awards, with a few exceptions such as the K awards, were reduced by 12%. On March 31, the President signed a continuing resolution effective until the end of the fiscal year; following this, 8% of the reductions were restored to these grants. Contracts experienced a more than 10% cut in the budget, from $11,078,000 in 1981 to $10,100,000 in 1982, requiring the phaseout of various contracts and short-term funding or reductions of others. Our payline, the worst of all institutes, remained at priority score 155 for a long time creeping up to priority score 158 only in the third trimester of the fiscal year. It is hoped that we will reach 160 by the end of the year.

We were able to pay only five competing K awards this year, among them the newly instituted Clinical Investigator (K08) awards. Budget for the conference grants was restricted to $80,000, roughly divided between the two major programs. The number of competing training grants awarded dropped as did the fellowships. Institutional allowances were reduced to half for trainees and to $3,000 for individual postdoctoral fellows. Several R01 applications, either solely or primarily assigned to NIAID, were negotiated by program staff and transferred to other BIDs because of our inability to fund those meritorious applications.

While the number of grants and contracts were reduced, the review workload of the chartered committees increased significantly. The Allergy, Immunology and Transplantation Research Committee reviewed 34 program project and Center applications (compared with 11 last year) of which 20 were site visited; the total review workload of this committee was 117 this year compared to 51 last year (see Table IV on Page 14-2). The Microbiology and Infectious Diseases Committee had a review workload of 55 compared to 33 last year.

This fiscal year marks the beginning of the waiver of the payback of the first 12 months of the trainee's total NRSA support, based on the NIH's interpretation of a recent amendment to the legislation governing the NRSA. (However, the Department is examining this issue and has commented that perhaps NIH has interpreted the amendment too liberally).

The Grants Management Branch was reorganized from a single branch to one with three formal sections, the IAID Section, the MID Section and a Grants Support Section. This was felt by the GMB chief to be a functionally more effective arrangement which would not require additional personnel.

Filling of some personnel vacancies has moved at a snail's pace. Clerical assistants to the Committee Management Assistant and the Assistant to the Acting Director, EAP have not been hired, and these busy offices have not had any clerical help for the past several months. There has been no active Acting Deputy Director, EAP for nearly a year. The Acting Director, EAP has had to manage the EAP without such help in addition to her other responsibility as Chief of the Program and Project Review Branch.

Predictably, as a consequence of the stringent budget, the number of grant applications processed increased and reached unprecedented proportions at the May Council. A dedicated and hardworking support staff saw the NIAID through the May Council without mishap.

On orders of the Director, NIAID, the position of training officer as well as the Financial Analysis Section and the Program Analysis and Evaluation Branch were moved to the Office of the Director.

B. Personnel

There were ten departures from the EAP, either from retirement, movement to other offices in NIAID or transfer to other BIDs. Dr. Sheffield, formerly executive secretary of the Allergy, Immunology and Transplantation Research Committee, moved to the Parasitology Program and was replaced by Dr. Nirmal Das, formerly Program Director of the Cell Biology Program at the NIA. Mrs. Margery Grubb has been serving as Committee Management Assistant beginning this fiscal year. When Dr. Louis Bourgeois retired in August of 1981, the position of training officer was transferred to the Office of the Director. Ms. Stesney, Assistant to the Acting Director, EAP, has assumed the duties of coordinating the R13s, formerly held by Ms. Warfield, in addition to her other duties. Ms. Warfield has moved to the Office of Program Planning and Evaluation.

The Financial Analysis Section and the Program Analysis and Evaluation Branch were transferred to the Office of the Director during this fiscal year.

Other personnel actions are moving very slowly. Ms. Grubb has not had an assistant since December 1981 and Ms. Stesney has no clerical assistant.

The Acting Director, EAP, has been functioning in that capacity since January 1981 in addition to her duties as Chief, Program and Project Review Branch.

FY 82

Full-Time Permanent Employees who have left EAP

Merlene Bagley
Linda M. Blazer
Lila J. Cave
Mary Lou Davis
Carol A. Deakin
Dorothy Leggin
Frances M. Schlademan
Harley Sheffield
William J. Stalters
Mildred S. Warfield

New Full-time Permanent Appointments

Patricia M. Coates
Nirmal K. Das
Judith N. Johnson
Cynthia R. McDermott
William O. Mitchell
Mary D. Shryock

Awards

None

C. Committee Management

While a new Committee Management Assistant came on board in August, 1981, the clerical assistant resigned in December and has not been replaced despite lengthy recruiting efforts.

Four members retired from the Council as of October 31. The five (one replacing an earlier resigner) new members did not come on board until early December due to a Department hiring freeze. The nomination slate for terms beginning November 1 has been prepared and is being reviewed by the Department.

Four vacancies on the Board of Scientific Counselors have been filled and invitations have been issued for members to replace two whose terms expired June 30.

Five new members for the Microbiology and Infectious Diseases Advisory Committee and four for the Allergy, Immunology, and Transplantation Research Committee (AITRC) began their official duties as of July 1. The nomination is still pending for a fifth new member of the AITRC.

The Department has initiated a new procedure for reporting and projecting the cost of operating chartered committees. A Financial Operating Plan (FOP) is required for each committee, based on calendar year operations with precise projected costs required for the upcoming year. These FOPs must be submitted with all charter renewal requests, as well as with other reports required by the Department.

D. Conference Proposals

Beginning with the September/October 1981 Council cycle, all grant applications submitted to the NIH for the support of scientific conferences, workshops and meetings have been handled by the BIDs, through the conference grant mechanism (R13). Where a mutual interest existed, DRG arranged for joint sponsorship of a conference by two or more BIDs through multi-Institute assignment. Initial review was conducted by the primary assignee. All R13 conference grant applications assigned to NIAID during FY 1982 were co-assigned to at least one other institute. During FY 1982, thirty-four conference grant applications with NIAID as the primary Institute assignee received initial peer review for scientific merit by one of the three NIAID chartered committees (Table II). Following each Council review, recommendations for NIAID funding contributions were made by Program staff to members of the Research Contract Advisory Group (RCAG) for their consideration. RCAG recommendations were then forwarded to the Director for final approval. In FY 1982, the amount of NIAID funds set aside for partial support of eighteen conference proposals will not exceed $80,000, or $40,000 for each of the two major programs. In order to facilitate timely issuance of conference grant awards, this entire process was carefully monitored by the NIAID conference grant coordinator Ms. Stesney. Actions taken by review, administrative and management staff, both within NIAID and the other BIDs identified as cosponsors by DRG, necessitated a continuous collaborative exchange of information among Institutes concerning all actions involving the receipt, assignment, review, and funding of these proposals. The NIAID conference grant coordinator serves as the focal point for all information about grant applications for the support of scientific meetings by the Institute.

TABLE II

NUMBER OF R13 CONFERENCE GRANT APPLICATIONS
REVIEWED BY NIAID, FY 1982*

	Institute Assignee		
	AI Primary	AI Secondary	TOTAL
Approved for Funding	17	1	18
Approved at Fundable Priority Not Approved for Funding	2	4	6
Approved at Unfundable Priority or Disapproved	15	2	17
TOTAL	34	7	41

*September 1981, January 1982 and May 1982 Councils

E. Transfer of Research Grant Applications to Other BIDs

During the past year there has been an increase in the number of applications either primarily or solely assigned to NIAID which were transferred to other institutes because of their ability to fund the proposal. This increase in transfers has been a reflection of this Institute's funding capability, and does not relate to the relevance of the proposed research to the NIAID's programmatic interests. The Acting Director of the Extramural Activities Program and the Institute's Referral Liaison have collaborated with Program staff in monitoring the status of applications which received priority scores falling beyond the Institute's payline. In this way we are best serving the interests of the principal investigator by negotiating transfers of assignment to the Institute most, capable of making the award. Implementation of transfers in a timely manner requires a cooperative exchange of information between involved NIAID extramural staff and our counterparts in other BIDs. Table III shows the number of applications transferred from NIAID to other BIDs for funding consideration in FY 1982.

TABLE III

GRANT APPLICATIONS TRANSFERRED TO OTHER BIDs FOR FUNDING CONSIDERATION, FY 1982

Awarding BID	Number
CA	16
GM	8
HL	4
AM	3
AG	1
HD	1
TOTAL	23

F. Study of Priority Scores and Study Section Behavior

In response to a recurring request by the NAAID Council, the Assistant to the Acting Director, EAP, has continued to observe the scoring characteristics of DRG Study Sections. Tabular statistical data illustrating Study Section scoring patterns and the effect of priority score versus percentile ranking have been presented to the last five Councils. Council members have responded to Dr. Krause's presentation of the information with continued and growing interest. Scoring patterns have been developed for ten Review Groups which have approved twenty or more NIAID applications per Council round (ALY, BM1, EVR, IMB, IMS, BM2, MBC, MG, TMP, VR). Deviation from the NIAID mean has been used as an indication of the degree to which these ten Study Sections are inclined to give priority scores of 175 or better to NIAID-assigned applications. A tabular presentation of this data shows a random fluctuation both vertically; i.e., from Study Section to Study Section, and horizontally; i.e., from Council to Council, over the five Council rounds. A supplementary study was prepared in response to a request from a Council member and focused on the linear (horizontal) correlation among the voting patterns. The technique of correlation analysis was applied to this information in order to determine any real differences in these variations. Preliminary data shows that the degree of correlation varies considerably among the ten Study Sections analyzed.

The second part of this continuing study involves a comparison of the use of priority scores versus percentile ranking in making funding decisions. There is little difference in the total number of applications which would be funded by either of the two methods. Obviously the difference lies in the specific grant applications which would move inside or outside of the payline. This comparison illustrates that our current method of identifying selected programmatically relevant applications within 50 points beyond the payline for "Selective Payment" is perhaps still the preferred method of payment.

I. CONTRACT MANAGEMENT BRANCH

The Contract Management Branch provides management services to the Institute's Research Program including solicitation, negotiation, award, and administration of all Institute research contracts.

The CMB continues to implement contract policies and procedures promulgated by higher procurement authority. The CMB works very closely with the Program and Project Review Branch and the various NIAID Project Officers and provides contract management expertise necessary in order to continue to have an effective contract program.

Through effective contract management, NIAID has continued to increase its percentage of competitive awards from 26.2% in FY '78 and 37.3% in FY '79, to 60% in FY '80, to 67% in FY '81, and an estimate of 75% in FY '82. NIAID is one of the few Institutes which exceeded the competitive goals established by Division of Contracts and Grants.

Other accomplishments for FY '82 are:

1. Led all Institutes in competitive and scheduling goals for FY 81/82 which are established by PHS.
2. Even distribution of contract awards within the fiscal year.
3. Made contract awards in all three socioeconomic programs; 8(a), Small Business Administration and women-owned organizations.
4. Led all Institutes (in total percentage) in meeting the goals established by Division of Contracts and Grants in closing out of contracts.

In FY '82, CMB received approval for 16 contract requests and issued about 549 requests for proposals to organizations which expressed interest. In response to the above advertisements, 59 proposals were received and 19 new competitive contract awards were made.

Due to the continuing requirements to stabilize the research project base and the reduction in the NIAID approved FY '82 contract budget, the contract budget has been reduced from $11,078,000 in FY '81 to an estimated amount of $10,100,000 in FY '82. The number of active contracts has been reduced from 120 in FY '81 to an estimated 105 in FY '82. To accomplish this reduction, various funding mechanisms were implemented which included delayed, short-term funding plus renegotiation reduction on on-going contracts; various contracts were phased out.

II. GRANTS MANAGEMENT BRANCH

Fiscal Year 1982 continued to reflect increases in grant activity for NIH, NIAID, and for the GMB. In the NIAID, Grants represent approximately 72% of the Institute's total budget, with over 1,700 grants for approximately $170 million awarded in FY '82 (up from $168 million in FY '81). The GMB is responsible for the fiscal and administrative management of all grants and awards issued by the NIAID. The GMB works very closely with program staff and provides the fiscal and administrative expertise necessary for effective program management. The GMB also serves as an interpreter of grant policy and procedure issued by the several echelons within the DHHS.

In reference to changes cited in previous annual reports, we regret to say that certain highly valued management tools have been rendered obsolete. The categorical Report of Expenditures, which was to be replaced by the non-categorical Financial Status Report, has in fact fully materialized. The loss of the categorical expenditures report deprives GMB of a significantly valuable management tool for monitoring grants. However, the change in the project-period concept that allows the "savings" from one project-period segment to help fund another continues to allow the institute to stretch the limited research dollar just a bit further.

Of potential impact on grants management activities in the future are four issues that have emanated from the NIH Directors Advisory Committee. They are:

- Extensions (Liberalization) of Institutional Prior Approval System

- Automatic Carryover of Unexpended Direct Costs

- Transfer of Costs Between/Among Related Projects

- Limited Trial of the Fixed Obligation Grant

Currently there are four working groups, each assigned the task of developing an NIH position on their respective issue. Each of these issues will bear very close scrutiny in the future.

The fact that we operated on a continuing resolution during FY 1982 posed many operational problems for the GMB. Many awards prepared early in the fiscal year had to be revised to reflect budget reductions necessitated by our operating at a level less than the expected full operating budget. Generally, for all research awards, the award level was reduced by four percent from the committed/approved level. Actions on other types of awards were as follows:

- Individual Fellowships (F32's) were prepared reflecting $3,000 for the Institutional Allowance (normally $5,000).

- Institutional Training Grants (T32's) were prepared reflecting allowances of $2,500 per postdoctoral trainee and $1,500 per predoctoral trainee (normally $5,000 and $3,000 respectively).

- Awards made for Salary only (Career-K4's and K6's) were not reduced.

Award notices prepared in FY 1982 did not reflect any reductions in future period commitments. However, it remains to be seen as to whether or not we will be able to honor those full commitments in FY 1983 and beyond. Further, with regard to FY 1983, it is anticipated that indirect cost awards, for research grants, may be made at the actual amount, less ten percent.

Again in FY 1982, staff of GMB were able to make many valuable contributions, both as members and chairpersons, of policy and procedure work groups, committees and subcommittees at the NIAID and the NIH. The Chief, GMB was Director of a new STEP Module entitled "Shrinking Research Dollars: Funding Issues, Mechanisms, and Alternatives." Additionally, the Chief, GMB participated as a faculty member at the "NIH Grants Administration Conference" held in Monterey, California and attended by representatives from more than one hundred grantee institutions.

During FY 1982, the GMB was formally reorganized from a single branch with no formal sub-structure to a single branch with three formal sections. The Branch, with the respective Section Chiefs, is comprised thusly:

SECTION	SECTION CHIEF
Immunology Grants Management Section	Mrs. Mattie Tynan*
Microbiology Grants Management Section	Mr. Todd Ball
Grants Support Section	Mrs. Marietta Robinson

*Mrs. Tynan also serves as the Deputy Chief, GMB

It is expected that the reorganization of GMB will further the objectives and better facilitate the significant interactions between and among Grants Management, Program, and Review. We have already experienced gains in FY 1982 and expect further advances in the future. Clearly, the relationship and partnership between GMB and PPRB has been heightened.

In FY 1982, as in the past, employees were encouraged to broaden their horizons beyond their day-to-day routine and fill spots created by temporary absences. Classes, seminars, and STEP programs were attended with more regularity. In general the staff of GMB continued to move forward in their individual development while maintaining a high level of excellence in pursuit of the day-to-day duties and responsibilities that encompass all grant programs funded by the NIAID.

III. PROGRAM AND PROJECT REVIEW BRANCH

A. Allergy, Immunology and Transplantation Research Committee, NIAID

The Allergy, Immunology and Transplantation Research Committee is composed of two subcommittees, the Allergy and Clinical Immunology Subcommittee (AIRC), and the Transplantation Biology and Immunology Subcommittee (TBIC). The AIRC reviews programs and projects in allergy, clinical immunology and immunopathology, while the TBIC is primarily concerned with programs and projects in immunobiology, immunogenetics, immunochemistry and transplantation biology. These committees provide initial review of grant applications and contract proposals for special programs which include program projects and centers, institutional training grants, conference grants and special developmental programs in the areas mentioned above. In addition, the subcommittees assess the progress, evaluate ongoing special programs of the NIAID and advises on program developmental activities in these areas.

B. Allergy and Clinical Immunology Subcommittee

Application/Proposal Review: The AIRC met three times during fiscal year 1982 and reviewed 55 grant applications which included 19 program projects and centers, 15 institutional training grants, 2 allergic diseases academic awards, 10 clinical investigator awards and 9 conference grants (Table IV). The total dollars requested in these applications and approved by the subcommittee were $39,664,634 and $14,755,283, respectively. The program project and center applications reviewed were in the areas of Mechanisms of Immunologic Diseases and in Asthma and Allergic Diseases. Nine of these applications were site-visited.

Program Advice: The program staff sought the AIRC's advice on a variety of issues including hybridoma banks, food allergy, drug allergy, NIAID task force on asthma, other allergic diseases and consensus development conferences in the areas of allergy and immunology that are of national importance.

Concept Clearance: The subcommittee cleared the concepts for the acquisition of allergens and immunologic reagents and the development of an RFA on occupational immunologic lung disease at a future date.

C. Transplantation Biology and Immunology Subcommittee

Application/Proposal Review: During their three meetings in FY 1982, the TBIC reviewed 40 applications which included 15 program projects, 8 conference grants and 17 contract proposals (Table IV). All grant applications were approved and total dollars reviewed and approved were $20,412,197 and $8,898,837, respectively. The program projects reviewed were in the areas of Lymphocyte Biology and Transplantation Immunology. Eleven of these program projects were site-visited. The 17 contract proposals were received in response to four RFPs: RFP-82-3, "Process and Prepare Alloantisera Specific for H2 Murine Cell Membrane Antigens," (one proposal); RFP-82-6, "Development and Clinical Evaluation of a Rapid Cellular Crossmatching Technique Suitable for Use in Cadaveric Organ Transplantation," (three proposals); RFP-82-7, "Screening, Characterization and Acquisition of Histocompatibility Testing

Transplantation Antigens of the Ethnic Subpopulations in the United States," (four proposals); and RFP-82-14, "Maintenance of an International Bone Marrow Transplantation Registry," (two proposals). Twelve of these proposals were approved. The dollars reviewed were $6,674,945. The NIAID Research Contracts Approval Group recommended five of these contract proposals for funding consideration at a total cost of $1,560,080.

Program Advice: Program staff sought advice from the TBIC on a variety of issues including hybridoma banks, serum banks, and bone marrow transplantation registry.

TABLE IV

REVIEW WORKLOAD OF THE AIRC AND TBIC, FY 1981 AND FY 1982

	AIRC REVIEW LOAD		TBIC REVIEW LOAD		AIRC & TBIC TOTAL REVIEW LOAD	
TYPES OF APPLICATIONS	FY 81	FY 82*	FY 81	FY 82	FY 81	FY 82
P01/P50	11	19	0	15	11	34
T32	10	15	0	0	10	15
K07	3	2	0	0	3	2
K08	0	10	0	0	0	10
R13	2	9	9	8	11	17
N01	4	0	1	17	5	17
Interagency Agreement	0	0	2	0	2	0
Program Concept Clearance	0	2	3	0	3	2
SUBTOTAL	30	57	15	40	45	97
Site Visits**	6	9	0	11	6	20
GRAND TOTAL	36	66	15	51	51	117

*One P50 and one T32 application from AIRC members were reviewed by a Special Review Committee

**Only new P50/P01 applications are site-visited.

D. Microbiology and Infectious Diseases Advisory Committee

The Microbiology and Infectious Diseases Advisory Committee (MIDAC) held three meetings: October 1981, March 1982 and June 1982.

Grants: The MIDAC reviewed one program project renewal, six new program project applications in response to an RFA on the "Epidemiology and Natural History of Genital Herpes," and one supplement to a program project application. All seven program project applications were site visited. They also reviewed 21 institutional fellowship applications and three applications for the Clinical Investigator Award. All of these applications were approved. Of the sixteen applications for conference grants, eleven were approved. The total dollars reviewed for grants were $7,280,389 and the total dollars approved were $4,806,357. Included in the totals was one institutional training grant application of a MIDAC member which was reviewed by an Ad Hoc Panel.

Contracts: The full MIDAC reviewed 28 contract proposals involving 5 RFP's and one unsolicited proposal. Eighteen proposals were approved and seven were awarded. The total dollars competitively reviewed by MIDAC for contracts was $3,631,848 and the total amount awarded was $1,410,328. The one unsolicited proposal was disapproved. Four ad hoc meetings were conducted to review 9 contract proposals to 3 RFP's and to one addendum to a contract. The three RFP's were for new and competing proposals. One proposal was awarded for each of the RFP's. In addition two sources sought reviews were held for which RFP's were subsequently issued.

Table V shows the workload of the Committee in 1982 compared with 1981.

TABLE V

REVIEW WORKLOAD OF THE MIDAC, FY 1981 AND FY 1982

	NUMBER OF APPLICATIONS/PROPOSALS REVIEWED	
	MIDAC 1981	MIDAC 1982
CONTRACTS		
Solicited (RFP Responses and Renewals):		
Full Committee	25	28
Ad Hoc	5	9
Unsolicited	1	0
Total Proposals Reviewed	31	37
GRANTS		
P01/P50		
Solicited (RFA Responses)	0	6
Unsolicited (renewal)	1	1
Supplements	1	1
T32	21	21
K08	0	3
Conferences (R13 & Fogarty)	10	16
PROJECT SITE VISITS (P50/P01)	0	7
PROGRAM EVALUATION	3	0
CONCEPT CLEARANCE (FULL COMMITTEE)	1	0
TOTAL PROPOSALS	33	55

E. REVIEW SERVICES UNIT

The Head of the Review Services Unit position, vacant since October 1980, was filled on November 1, 1981. The Unit continues to be understaffed with a need for permanent personnel who can be trained to assume duties and have a knowledge of the flow of the material and the scope of the work. There has been a moderately rapid turnover of personnel. In addition, a lack of space remains a critical problem.

The workload has been heavy during the past two Councils. May Council was especially heavy because summary statements were not received from DRG in a timely manner and RSU had to copy many summary statements. Frequent breakdowns of the xerox machine in face of the heavy demands aggravated the situation. However, with the efforts of RSU staff, help from other NIAID support staff, and the cooperation of other institutes which have allowed us to use their copy machines, Council preparations were completed on schedule.

IV. RESEARCH RESOURCES SECTION

A. Special Activities

The Acting Director, NIH approved the disestablishment of the Research Resources Branch and the establishment of the Research Resources Section effective February 24, 1982. Mrs. Sylvia Cunningham has continued to serve as Acting Head of the operations. In addition to the Head, the RRS is supported by one clerical position. A replacement for this position is currently being recruited. The RRS is responsible for the management of operations including distribution and cataloging of the microbial, allergen and some of the immunologic reagents.

The need for well-characterized reference reagents as an adjunct to research is well recognized, and in support of the concept, the RRS conducts a program which distributes the reagents with information and technical advice on their characteristics and uses.

This year the Section once again made arrangements to take an Institute exhibit to the American Society for Microbiology (ASM) meeting held in Atlanta, Georgia, March 7-12, 1982. Due to budget restrictions, the exhibit was located in the scientific area rather than the technical/commercial area. The location of the scientific exhibits was ideal in that it was in the center of the technical exhibits. The actual exhibit was a portable one containing six translucent drawings of the various Institute activities. Use of this type of exhibit reduced the expense considerably in that the actual equipment for the exhibit was carried as luggage and assembled by the one person who attended the meetings to be with the exhibit. The exhibit was well received and a great deal of interest was shown by the many meeting attendees.

B. Allergen Reagents

Under a procurement contract with the American Type Culture Collection, the Section is now making arrangements to have processed and packaged a lot of complement component C3 and one additional lot of the major penicillin product. The penicillin products have been utilized during the past year by six NIAID contractors who are conducting clinical studies to determine the penicillin sensitivity of patients.

The Section will also arrange for the processing and packaging of five additional lots of hypersensitivity pneumonitis antigens and one antisera as soon as the bulk testing has been completed by the allergen certification laboratory (Mayo Clinic). It is expected that this task will be completed prior to the end of this fiscal year.

Since award of the contract to Mayo to serve as the allergen reference laboratory, there has been close coordination of activities between this Section and the contractor in order to schedule packaging, labeling and the cataloging of various items such as the food preparations, a new lot of cat pelt extract and the other items previously mentioned.

In addition to the activities listed above, the RRS has continued to distribute allergenic products such as ragweed antigen E, K and Ra3, rye grass I, II and III, venoms of honey bees, yellow hornets, white-faced hornets, yellow jackets and hymenoptera venom; these venom products are available for distribution under an IND as well as for *in vitro* use. The supply of Antigens E and Ra3 has almost been depleted; distribution of these items has been stopped. The remaining supplies are being held to be compared with the new lots being produced by the Rockefeller University. Production of Antigen E has been completed and is now being tested. As soon as the test results have been analyzed, the material will be processed and packaged. Production of Ra3 is scheduled for completion in September.

C. Immunological Reagents and Resources

The contract awarded in FY 1978 to Flow Laboratories for the maintenance and breeding of rabbits of known genotype for use in immunological studies was terminated for the convenience of the Government effective March 19, 1982. This contract activity was merged with another facility (Spring Valley Laboratories) which maintains another rabbit colony. These facilities provide reference quantities of reagents and limited numbers of rabbits to responsible investigators doing immunological studies. During this period, 2 shipments of rabbit antisera and 7 shipments consisting of 49 live rabbits were made to various investigators.

D. Microbiological Reagents

Under a procurement contract with the American Type Culture Collection, arrangements were made to process the immune ascitic fluids of African swine fever, rift valley fever, African horsesickness and bovine ephemeral fever, prepared under an interagency agreement with the Plum Island Animal Disease Center. The four ascitic fluids will be placed in the Catalog as soon as the final test data have been reported.

The Enterovirus Typing Pools A thru H originally prepared in 1971 have been distributed widely and after 11 years use, the supply of these pools is almost exhausted. A request from WHO for the pools had to be denied because of the low level of material available. Additional bulk materials are available to have another batch prepared; however, certain preliminary work would be required; i.e., testing and evaluation, processing and packaging, and retesting of final packaged product. In view of the fact that the Institute has assigned a low priority to maintaining reagents primarily for surveillance and epidemic investigations and also because of budgetary reductions, the American Type Culture Collection (ATCC) was asked if they would be in a position to assume responsibility for preparing a new batch if the Institute provided the bulk material. The ATCC has responded that they would be interested in preparing new pools but would have to have additional financial support in order to do so. The WHO has been contacted to determine if they would be willing to contribute funds for this project.

E. Research Resources - Research Reagents

While responsibility for the actual production of reagents is with the various Institute program areas, RRS has continued to sponsor certain service-type contracts which serve the needs of the various program elements. Upon notification of any problem from investigators using any item distributed by this office, arrangements are made to have the product tested by a competent laboratory.

The intensive work of prior years has resulted in the completion of work on the enterovirus, adenovirus, rhinovirus, myxovirus and the agents and antigens of hepatitis A and B. In most cases, seed virus preparations and corresponding antisera are now available for most of the viruses of public health interest. The reagents which have resulted from the various projects have been most useful, particularly the hemagglutinins, neuraminidases, ribonucleoproteins and the influenza viruses of man and animals.

After a category of reagents has become established, and the area of research that it supports is well defined, it is of questionable value for RRS to continue storing and distributing that category of reagents for a prolonged period of time. During FY 1975, it was determined that four viral reagent groups fell into that category. The RRS therefore, entered into a five-year contractual arrangement with the American Type Culture Collection (ATCC) to transfer the enterovirus, adenovirus, rhinovirus and arbovirus reagent collections to the ATCC whose prime function is to store and distribute these type of reagents. Additional reagents are currently being identified for possible transfer to ATCC.

F. Processing and Distribution

In addition to the various program elements detailed above, the RRS also distributes coronaviruses, herpes viruses, interferons, mycoplasmas and reoviruses.

During this year a blanket type procurement agreement has been in effect for the processing and packaging of various items. To date four arbovirus ascitic fluids, one penicillin lot and complement component C3 have been assigned to completion under this arrangement with the American Type Culture Collection.

The RRS reagent collection consists of over 350 individual reagents. The repository and distribution contract remains at Flow Laboratories, McLean, Virginia. A tabular record of distribution by the facility since FY 1974 follows:

TABLE VI

DISTRIBUTION OF VIRAL, MYCOPLASMAL, AND ALLERGEN REAGENTS

Fiscal Year	Total Transactions	Total ampoules & vials distributed
1974	500	9,932
1975	592	6,751
1976	762	10,188
1976 (TQ)	192	3,126
1977	613	7,633
1978	605	6,851
1979	782	16,223
1980	793	11,238
1981	912	11,096
1982	599	8,920*

*Projected total for 1982. Actual figure as of 7/15/82 is 6,690

OFFICE OF THE SCIENTIFIC DIRECTOR, NIAID

1982 Annual Report

Table of Contents

PAGE

16-1 Summary of Program--Sell

SUMMARY OF PROGRAM
Laboratory and Clinical Research, NIAID
October 1, 1981 - October 1, 1982

Office of the Scientific Director

The annual report of the Intramural Research Program contains individual summaries of the research projects in the 13 laboratories which constitute the research components of the National Institute of Allergy and Infectious Diseases. Administrative responsibility for the Intramural Research Program resides with the Office of the Scientific Director (OSD). Ten of the laboratories are located at the NIH campus in Bethesda. The three remaining laboratories are located in Hamilton, Montana, also called the Rocky Mountain Laboratories.

The administrative functions of OSD are the responsibility of Mr. Charles Criswell at the Bethesda campus and Mr. Robert Steiner at the Rocky Mountain Laboratories. Recent addition of facilities in Building 550 at the Frederick Cancer Research Center have provided space for components or sections of several laboratories of the Intramural Program. Mr. Stan Nagle provides the administrative functions at Building 550 on the FCRC campus.

Recruitment of new personnel to the Intramural Program of NIAID has been particularly gratifying during the past year. The initiation of new programs in molecular biology have been successfully undertaken to provide this new approach, both to basic problems of immunology and infectious diseases. The Laboratory of Infectious Diseases now has two senior investigators, Dr. Chin Chu-Lai and Dr. Venkatesan, each with special expertise in molecular virology and applying their talents to the study of respiratory viruses and enteric viruses. Rapid progress is being made with this new technology which provides the hope for structured and specific modification of viral genomes so as to provide new viruses potentially more suitable for vaccine development. Molecular biology has also been introduced into the immunology laboratories with Dr. Ed Max providing a focus for molecular biology in the Laboratory of Immunogenetics and Dr. Ron Germain has been recruited to establish a similar program in the Laboratory of Immunology. Dr. Germain's capability comes to a laboratory which only in the past year utilized the talents of Dr. Mark Davis to approach the cloning of genes expressing differences between cells such as the T-helper and T-suppressor cells or the T-cell and the B-cell. This work is moving rapidly and providing a unique opportunity to understand immunological development and immunoregulatory control. We have also been able to recruit a senior scientist, Dr. Warren Strober, to join the Laboratory of Clinical Investigation and establish a new section on mucosal immunity. As over 80 percent of all human infections occur at mucosal surfaces, this new laboratory will have special impact on our programs which interrelate our responsibilities in infectious disease research with those studies of the immune system and its regulation.

The Rocky Mountain Laboratories continue to prosper. A major renovation of a laboratory for electromicroscopy was completed this year, and Dr. Claude Garon who transferred from the Bethesda campus to the Hamilton campus last year has initiated an active program in collaboration with scientists of all three of the research laboratories at RML. With the rapid redevelopment of research programs at RML, there has been considerable stress on the administrative capabilities and central service functions under the supervision of Mr. Robert Steiner, Chief of the Operations Branch at RML. Nevertheless, the Operations Branch continues to provide services and perform minor and even major renovations with remarkable skill and competence.

The Editorial Office of the Intramural Program continues to work very effectively. We have expanded the editorial system to include seven new editorial assistants who work with the Editorial Office to provide for preparation of manuscripts and professional editing and review of all publications with a careful bibliographic review of all references. During the year, word processing equipment was obtained and made available to all members of the editorial staff, as well as the senior secretarial staff of each laboratory. This new mini computer linked operation provides for the most rapid and accurate implemention of the editorial review of manuscripts. The number of manuscripts in the Intramural Program has grown rapidly and now exceeds 450 per year. The quality of the manuscripts continues to improve with continued compliments from the intramural scientists, as well as several letters of commendation from journals who are recipients of the manuscripts.

For the forth successive year, we have held the Biomedical Science Program during which 50 students were invited to NIH to spend several days with scientists in their laboratories. During this period, they had an opportunity to interact and learn of work opportunities at NIAID, as well as other institutes at NIH. This program, which is geared to minority students, is directed by Dr. Katherine Cook-Jaouni who provided the leadership and management of the program. Despite fiscal restraints, we were able to provide work opportunities for 60 students of whom over Over two-thirds of the summer students were either minority or female or both. We do continue to have a shortage of Hispanic and Native American employees in all grade levels. There is also a continued shortage of all minorities and women in grade levels GS-13 and above. This is in contrast to grade levels GS-11 and below where minority participation exceeds that expected either by review of national or local statistics. We are anticipating that our summer minority recruitment and training program will begin to yield graduates of Ph.D. and M.D. programs who can join the institute as post-doctoral scientists in coming years.

The Animal Care Facilities of NIAID continued to improve. The full accreditation was received for the Rocky Mountain facilities and AALAC recommendations for animal facilties at Building 550 at the FCRC campus as well as for the animal facilities at the NIH campus are being implemented so that full accreditation is expected by the end of FY 1983. During the year plans were made to move a major portion of the animal facilities into a newly renovated building, 14B, which was constructed so as to meet all requirements for AALAC accreditation. Approximately 9,000 square feet of animal space will be available in 14B and will be used to house animals which are being dislocated from Building 5 by the "round-robin" renovation of older science buildings on the NIH campus which require the transfer of scientific personnel from Building 8 to Building 5. Dr. Peter Golway serves as the senior veterinarian and continues to provide outstanding support for the development of veterinary services. Mr. Carter Smith is the Chief Animal Caretaker and has been responsible for implementation of a major reassignment of animals in animal care spaces as the moves to 14B are undertaken. It is anticipated that these moves will be completed in FY 1983.

The P-4 high containment facility in Building 550 at the FCRC campus has been recertified and has been made available for U.S. scientists' collaboration and participation. Dr. Murphy of Harvard University will be using this facility for the cloning of diphtheria toxin into E. coli, an experiment which not only has high scientific merit, but should provide for a new level of understanding of risk assessment. P-4 biocontainment has also been made available on the NIH campus in Building 41T. Currently the building is occupied by Dr. Gordon Wallace of NIAID, who is studying the virology, epidemiology and biology of ectromelia (poxvirus) infections in mice. This problem in recent years has caused a loss several millions dollars at the NIH campus and provided a threat to all research in the United States involving mice. Dr. Wallace is making rapid progress in the understanding of the epidemiologic spread disease, leading hopefully to better methods for containment, treatment and prevention of ectromelia in mice.

The "round-robin" renovation of older buildings on the NIH campus is continuing. Spaces in Building 5 have been renovated during this fiscal year, and these renovations will be completed in December of 1982. In early 1983 it is anticipated that all NIAID scientists now located in Building 8, will be relocated to newly renovated spaces in Building 5, spaces which were made available and vacated through the movement of animals to Building 14B.

New central service facilities have been established in NIAID to assist the scientists. These include the acquisition of equipment for peptide synthesis and the assignment of an expert to provide peptide synthesis capability for both immunological and virological investigations. In addition, a DNA synthesizer (gene machine) has been acquired and is now available for preparation of gene sequences, both for direct investigation of viral gene control and to provide gene sequences for recombinant DNA insertion in E. coli and other hosts to provide biological synthesis and production by peptides.

The Ambulatory Care Research Facility was completed this year and NIAID has now fully occupied the laboratory research portion of its spaces on the 11th floor. The spaces have been provided primarily to the clinical allergy research program and to the clinical parasitology program involving those areas of parasitic infection which have an interrelationship with the hypersensitivity research of the allergy program. To this date, however, the clinical outpatient facilities of the Ambulatory Care Research Facility must be shared with other institutes so that the planned expansion of clinical investigation programs involving outpatients has not yet been possible.

Safety continues to have high priority in the Intramural Program of NIAID. NIAID was commended this year for its steady improvement in safety in all of its facilities at the Bethesda and Rocky Mountain campuses.

The scientific activities of the Intramural Program continue to be outstanding. Individual scientists have received recognition for their work, and peer review by the Board of Scientific Counselors continues to reassure us regarding the high quality of the scientific effort of NIAID. The recent selection of Dr. William Paul to the National Academy of Sciences gives further evidence of the scientific level of performance of the Intramural Program. Despite fiscal and budgetary constraints, the Intramural Program has moved rapidly to utilize the new technologies of hybridoma fusion and recombinant DNA methodology to maintain its position on the frontiers of science as related to immunology and infectious diseases. Budgetary constraints do provide some concern, however, as this year for the third straight year we have been unable to increase the amount of funds available for "other objects" in support of laboratory research. It is hoped that in fiscal years 1983 and 1984 some relief will be obtained so that the laboratories may be more adequately supplied with the materials necessary to perform their research.

LABORATORY OF BIOLOGY OF VIRUSES

1982 Annual Report

Table of Contents

Z01 AI
Project Number

SUMMARY 17-1

00123-16 Structure and Replication of Poxvirus Genomes 17-12

00124-13 Replication of Viral Specific RNA in a Rat Nephroma Cell Infected with the Parvovirus KRV 17-13

00126-09 Functional Analysis of Vaccinia Virus DNA 17-14

00290-01 Site Specific and Deletion Mutants which Regulate SV40 Transcription 17-15

00291-01 Interaction of RNA Polymerase II with Defined DNA Templates 17-16

00292-01 Protein Factors Required for Specific Transcription 17-17

00293-01 Characterization of Proteins Associated with the Parvovirus, KRV, and its DNA 17-18

00294-01 Structure and Function of Adenovirus DNA 17-19

00295-01 Helper Factors Required for Expression of the Adeno-Associated Virus Genome 17-20

00296-01 Characterization and Production of Parvovirus Proteins 17-21

00297-01 Mechanism and Regulation of Adeno-Associated Virus DNA Replication 17-22

00298-01 Vaccinia Virus: An Infectious, Selectable, Eukaryotic Cloning Vector 17-23

00307-01 Regulation of Vaccinia Virus Gene Expression 17-24

LABORATORY OF BIOLOGY OF VIRUSES

1982 Annual Report

Table of Contents

Z01 AI
Project Number

SUMMARY 17-1

00123-16 Structure and Replication of Poxvirus Genomes 17-12

00124-13 Replication of Viral Specific RNA in a Rat Nephroma Cell Infected with the Parvovirus KRV 17-13

00126-09 Functional Analysis of Vaccinia Virus DNA 17-14

00290-01 Site Specific and Deletion Mutants which Regulate SV40 Transcription 17-15

00291-01 Interaction of RNA Polymerase II with Defined DNA Templates 17-16

00292-01 Protein Factors Required for Specific Transcription 17-17

00293-01 Characterization of Proteins Associated with the Parvovirus, KRV, and its DNA 17-18

00294-01 Structure and Function of Adenovirus DNA 17-19

00295-01 Helper Factors Required for Expression of the Adeno-Associated Virus Genome 17-20

00296-01 Characterization and Production of Parvovirus Proteins 17-21

00297-01 Mechanism and Regulation of Adeno-Associated Virus DNA Replication 17-22

00298-01 Vaccinia Virus: An Infectious, Selectable, Eukaryotic Cloning Vector 17-23

00307-01 Regulation of Vaccinia Virus Gene Expression 17-24

The Laboratory of Biology of Viruses has as its goal an understanding of how viruses replicate and how they produce cytopathic effects within the cells that harbor them. Entry of viruses into cells can produce several different effects. In most cases, the ultimate consequence of a viral infection is cell death and lysis. However, this effect is the finale and there is a precisely orchestrated series of reactions that precede it. The virus is likely to have one or a few primary targets within the cell whose inhibition produces a cascade of other inhibitory processes and finally cell death. In other cases, the virus establishes a benign relationship with the cell and may integrate within the cell's DNA where its replication now is controlled by cell regulatory processes. However, its presence within the cell results in synthesis of new proteins that are specified by the viral DNA, and these proteins can impart new properties to these cells. Cellular processes which regulate cell growth may no longer function properly because of the presence of virus specified proteins.

Regardless of whether viruses alter cells which serve as their hosts, or kill the cells that harbor them, viruses require cells if they are going to replicate. Since virus growth is coupled to and dependent on cellular proteins, the viruses serve both as probes and provide models to describe normal cellular processes.

The past several years have seen a significant change in the focus of work within LBV. Recombinant DNA technology and DNA sequencing techniques have permitted the examination of conceptionally new problems. For most of the small viruses, precise knowledge of virion structure has been available but recombinant methodology has allowed detailed analysis of regions of the larger viruses to be defined. Using data obtained by DNA sequencing, information on viral specified RNA can now be compared with the DNA template from which it was copied. This has provided an understanding of how simple genomes can generate multiple viral proteins by using alternate splice sites to form overlapping but unique messenger RNA molecules. The precise localization of the start sites for transcription of specific genes has permitted the identification of regions that lie upstream from the start sites which regulate the rate of transcription. The localization of these control regions now permits investigators to enhance or suppress expression of specific genes by introducing site specific mutations into these control regions. These new approaches to studies with viruses are currently all in use in LBV. It is certain that they will provide an understanding of processes that regulate growth of viruses and of the cells that serve as their hosts.

Some of the highlights of this year's research efforts are detailed below.

Papovaviruses

Factors Required for In Vitro Transcription of SV40

Using HeLa whole cell extracts, *in vitro* transcription of SV40 DNA is inhibited when the DNA concentration is increased above an optimum level. This inhibition is reversed when histones H_2A, H_2B, H_3 and H_4 are added to the transcription system. Spermine and histone H_1 are not able to restore activity to the extract, and histone H_4 causes only a minor restoration at any of the concentrations tested. Maximum amounts of specific transcripts are observed at a DNA to histone ratio of 1.0 to 1.5. Single strand specific nuclease mapping of the *in vitro* transcribed RNA from the late promoters of SV40 shows that RNAs initiate from the same sites when transcription occurs either in the presence of optimum concentration of DNA or in the presence of inhibitory concentrations of DNA plus added histones. Micrococcal nuclease digestion studies of the DNA in the transcription system showed that at an optimal concentration, the DNA is protected from nuclease digestion. The same nuclease digestion pattern is observed in the presence of optimum DNA concentration and in the presence of inhibitory concentration of DNA and histones. If the DNA concentration is increased and transcription is inhibited, the DNA is more rapidly digested. The two major late promoters can be switched on by histone addition while the expression of the minor promoters is not affected by varying DNA concentrations or by histone addition. These results indicate that recognition of specific major late promoters of SV40 occurs when the DNA is complexed with proteins present in the Manley extract and the transcription complex requires a defined ratio of protein to DNA.

Recognition by Purified RNA Polymerase II of Start Sites for Transcription

In previous studies using purified RNA polymerase II and SV40 DNA Form I, it was shown that initiation of RNA synthesis occurred at internal sites on the DNA. Localization of the specific sites where initiation of RNA synthesis occurred *in vitro* showed that the conformation of the DNA drastically affects the selection of promoters by mammalian RNA polymerase II. Some of the major initiation sites were found to be located in close proximity to the promoters utilized *in vivo*. We have mapped these start sites more precisely using the purified enzyme or a cell free Manley extract as an enzyme source in order to transcribe the DNA. The Manley system has been shown to initiate transcription at the same start site as is used *in vivo*. We find the purified enzyme starts synthesis 30 nucleotides before the start used in the Manley extract. This start site is in the TATA control region first described by Goldberg and Hogness.

Site Specific and Deletion Mutants which Regulate SV40 Transcription

A series of mutants have been constructed containing base substitutions centered 25 nucleotides upstream from the major late SV40 RNA initiation site, map position 325. Using an *in vitro* transcription system which allows RNA polymerase II to accurately transcribe DNA, the transcriptional efficiency of wild-type and mutant templates were compared. The efficiency of promoter usage was determined by incorporation of a-^{32}P-UTP into transcripts that extend from the site of initiation to a termination site created by restriction endonuclease cleavage and by S1 analysis of RNA using probes labeled at their 5' termini. These experiments have identified a DNA sequence, 5'-G-G-T-A-C-C-T-A-A-T-T-3' (map position 294-304), located 21 to 31 nucleotides upstream from the initiation site, which in part controls the level of RNA expression.

Mutants pSVs-3 and pSVs-17, which had common C to T base substitutions at map positions 298 and 299, increased initiation of late RNA synthesis at map position 325 by five to tenfold. Mutants pSVs-7 and pSVs-24, which had common G to A base substitutions at map position 294 and 295, decreased RNA initiation at map position 325 by two to threefold. The base mutations did not affect RNA synthesis from the early SV40 promoter located at map position 185.

Transcriptional analysis of deletion mutant pSVs-KB6 also demonstrates the importance of the upstream sequences in the control of major late SV40 RNA initiation. Deletion of nucleotides 295-298 drastically reduced specific RNA initiation at nucleotide 325 in the *in vitro* transcription system. Comparative S1 analysis of *in vivo* RNA, isolated 36 hours post-infection with wild type and pSVs-KB6 virions demonstrates that the four base deletion not only decreased RNA initiation at nucleotide 325, but increased RNA initiation at three alternate sites located approximately 125 nucleotides upstream from the major RNA initiation site.

The identification of promoter control elements for the late SV40 gene is of particular interest in the study of eukaryotic gene regulation. The papovavirus late gene is a notable exception to the universal presence of a TATA box upstream from the mRNA start site. Thus, it is important to determine if other DNA sequences functionally substitute for the TATA box or whether a second class of promoter control elements exists in eukaryotic genes.

Parvoviruses

Comparison of Structural Proteins in Two Adeno Associated Virus (AAV) Serotypes.

It was previously demonstrated that adeno-associated virus (AAV) contains three structural proteins designated A (~ 90,000 daltons), B (~75,000 daltons), and C (~60,000 daltons). The composition in virions, which also reflects *in vivo* synthetic rates, is roughly 90% C with comparable amounts of A and B (about 5% each). Purified AAV virions types 1 and 2 labeled with ^{35}S-methionine were isolated by cesium chloride gradient centrifugation and electrophoresed in SDS polyacrylamide gels. It was found that slight differences exist between the corresponding proteins of AAV1 and AAV2. Proteins A and C of AAV1 are slightly smaller than A and C respectively of

AAV2 while B of AAV1 is slightly larger than B of AAV2. However, the relative amounts of A, B, and C are similar in AAV1 and AAV2. In addition, supernumerary bands of C (i.e., four distinct subspecies of C) are present in both AAV1 and AAV2. These findings indicate some common control feature in the genomes of both AAV1 and AAV2 which results in very similar overall protein structure although there is little or no antigenic relatedness between AAV1 and AAV2 proteins. Comparison of proteolytic patterns of peptide C from AAVs 1 and 2 were made after partial proteolysis by staphylococcal protease V8. The C proteins of AAV1 and AAV2 each yielded at least twelve reproducible breakdown products ranging in molecular weight from about 8,000 daltons to 40,000 daltons. The smaller products were more prominent at higher concentrations of protease V8, indicating that the larger products are probably only partially degraded. However, none of the proteolytic products of AAV1 comigrated with any AAV2 products, and the overall pattern of breakdown was distinctly different between the two viruses. Staphylococcal protease V8 digestion of AAV2 proteins yielded at least ten breakdown products ranging in size from 8,000 daltons through 20,000 daltons which were identical in peptides A, B, and C. There were two bands that appeared in the digestion of C but not A or B. There was a band that appeared in A and C but not in B. These findings indicate that all three proteins, A, B, and C, are translated in the same reading frame.

Helper Factors Required for Expression of the Adeno-Associated Virus Genome

The functions required for AAV replication that is provided by adenovirus (Ad) have been shown to be associated with several early Ad gene products. To investigate AAV transcriptional and translational requirements for Ad gene products, a duplex AAV DNA segment was cloned in pBR325 (pLHI). When cells were transfected with this plasmid, transcripts were produced which were capable of directing *in vitro* synthesis of the three AAV structural polypeptides, although *in vivo* synthesis of AAV polypeptides could not be detected by immunofluorescence or immunoprecipitation. Cotransfection with pLHI and intact Ad DNA enchanced the level of cytoplasmic AAV transcripts, and *in vivo* synthesis of AAV proteins was readily demonstrable. It was found, however, that combined transfection with pLHI and a recombinant plasmid that contained the Ad DNA-binding protein gene (pDPB) also enhanced the level of cytoplasmic AAV transcripts but did not promote *in vivo* AAV protein synthesis. When another plasmid that contained the Ad VAI RNA gene (p2VA)) was cotransfected with pLHI and pDBP, *in vivo* AAV polypeptide synthesis occurred. pLHI plus p2VA, however, did not induce *in vivo* AAV protein synthesis. We conclude that Ad DNA-binding protein augments the level of AAV cytoplasmic transcripts, whereas translation of these transcripts requires Ad VAI RNA. Furthermore, based on these and other experiments, it appears likely that DNA-binding protein also plays a role in the translation of viral mRNA, and that this activity may require a direct interaction with VAI RNA. These findings indicate the existence of a mechanism which regulates the expression of certain viral proteins with a high degree of specificity. In all probability, a counterpart(s) of this mechanism operates in normal cells, providing an analogous capability for regulating synthesis of particular cellular proteins.

In Vitro Synthesis of AAV DNA

AAV DNA synthesis *in vivo* is initiated on single-stranded genomic templates by a self-priming mechanism. Subsequent elongation yields a unit length hairpin intermediate. A second round of self-primed synthesis displaces the 5'-ended arm of the hairpin and leads to either (i) displacement of a complete plus or minus progeny strand (by virtue of a processing/synthesis step at the closed end of the hairpin) or (ii) concatermeric molecules if closed end processing/synthesis does not occur. These latter molecules can be eventually processed to unit length templates which, in turn, may also yield progeny strands by new self-primed rounds of displacement synthesis. The specific enzymatic and regulatory factors (both cellular and viral) that participate in AAV DNA synthesis are not clearly defined. To help identify and characterize these elements, two *in vitro* DNA synthesizing systems that generate AAV DNA replicating forms which correspond to those found *in vivo* have been examined. The systems are derived from nuclear extracts of Ad-AAV-infected, Ad-infected or uninfected KB cells. One system requires endogenous AAV DNA templates, the other requires exogenously added, denatured AAV virion DNA. Nuclear extracts are separated into two fractions, a sedimentation fraction (template fraction if derived from Ad-AAV-infected cells) and a supernatant fraction (polymerase fraction). Both fractions are required for synthesis on endogenous templates, whereas the exogenous template system utilizes only the supernatant fraction. Two distinct and potent inhibitors of DNA synthesis on single-stranded AAV templates have been extensively purified from KB cells. Although the inhibitors of DNA synthesis currently under study cannot be thought of as requirements, such factors may play important roles in regulating *in vivo* synthesis of both viral and cellular macromolecules.

The Characterization of Proteins Associated with Kilham Rat Virus (KRV) and its DNA.

During the replication of KRV in the rat nephroma cell line, the Rf form of KRV-DNA has been found to be associated with one major protein. The KRV-Rf DNA is isolated from a lysate of infected cells and isopycnic centrifugation in cesium chloride separates the viral DNA-protein complex at a density of 1.68 g/ml from other DNAs and proteins. The DNA-protein association is resistant to treatment with 1M NaCl and thus, the linkage is believed to be covalent. If the complex is treated with DNase I and spleen phosphodiesterase, the associated protein has a molecular weight by polyacrylamide gel electrophoresis of about 68,000. The linkage between the DNA and protein is a phosphodiester linkage. By labeling this phosphate linkage with ^{32}P, we were able to determine that the terminal amino acid in the linkage is phosphoserine. Proteinase digestion of the complex revealed that the terminal nucleotide is thymidine or cytidine. Restriction enzyme digestion of the complex electrophoresis of the fragments and comparison of the fragments to those from uncomplexed KRV showed a difference in mobility of both 5' termini of the DNA.

Replication of Kilham Rat Virus (RV) RNA in Infected Rat Nephroma Cells.

Transcription and protein synthesis of the viral RNA in the KRV infected cell and uninfected cell and also in a cell free system has been examined. Utilizing DNA-RNA hybridization and the techniques of Northern and Southern blotting from electrophoresis gels to membrane papers, we have identified at least five species of viral RNA. The RNAs are transcribed from the infected viral DNA and have the approximate lengths of 4.7, 3.4, 3.0, 1.25 and .95 kilobases (Kb). These RNAs are probably functional messages since they were isolated from the polysomal fraction of the cell. The 4.6 Kb RNA would represent a transcript of 95 to 100% of the viral genome. The most abundant message, about 3.1 Kb, represents over 50% of the viral genome and probably codes in the reticulocyte transcribing system for the most abundant viral protein (mw 68,000) which is the main viral capsid protein. This abundant RNA has an origin of transcription about 3.8 to 4.2 map units from the 3' end of the single-stranded KRV genome. The purified viral polysomal RNAs, when placed in a reticulocyte *in vitro* translation system, synthesize the known viral proteins in the virus capsid.

To study transcription and translation in more detail, we have developed a cell free system of isolated nuclei which synthesizes the five or six viral specific RNAs found in the *in vivo* infection. We have determined that the viral RNAs are polyadenylated on the 3' terminus and are transcribed solely from the viral strand of the virion, not from the complementary strand of the replicative intermediate. The 5' terminus of the RNA was shown by paper chromatography to be capped *in vitro* by the sequence $m^7G(5')ppp(5')A$. Incorportion of $[\beta\text{-}^{32}P]$ ATP into the 5' cap sequence suggests that initiation of viral RNA synthesis may occur in the infected isolated nuclei. Since the major 4.6 kilobase RNA is synthe-sized abundantly in the nucleus, we hope to find out if this is a single transcriptional unit and the other viral RNAs are multiple spliced transcripts.

Adenoviruses

Structure and Function of Adenovirus DNA.

It was previously demonstrated that DNA molecules extracted from adenovirus (Ad) particles (linear duplexes with molecular weights of 20-25 X 10^6 daltons) contain an inverted terminal repetition (ITR). The molecular arrangement of the repetition was initially determined by direct visualization of "panhandle" projections on circularized single-stranded Ad18 DNA. More recently, others have analyzed ITRs in the genomes of Ad types 2, 3, 5, 7, and 12 by nucleotide sequencing. For the major infectious components of encapsidated DNA, the lengths of these repeats have been found to vary from 103 bases (Ad2) to 162 (Ad12). In the case of Ad18, however, DNA molecules recovered from the main band of CsCl-purified virions possessed occasional ITR variability, with some repetitions approaching 20% of genome length. Furthermore, increases in ITR length were accompanied by equivalent inward deletions of adjoing sequences, so that total molecular length remained nearly constant. Although it is probable that molecules with inward deletions are defectives, it is not known for certain whether any molecule with an ITR is actually infectious (e.g., the repeat is always associated with a critical, small inward deletion), or, if infectious genomes do possess an ITR, whether ITR length can vary. To answer this question, Ad18 DNA from two plaque isolates (P-1 and P-2) derived from the prototype strain was analyzed by cleavage with restriction endonucleases and by electron microscopic heteroduplexing techniques. Fragment sizes were determined by contour length measurements, sucrose sedimentation and agarose gel electrophoresis. A physical order of fragments was obtained by comparative digestion of isolated fragments and heteroduplex mapping. End fragments were identified by their susceptibility to exonuclease digestion and by the presence of covalently attached terminal proteins. It was found that P-2 DNA molecules were about 4% longer than P-1 molecules, and that the observed length difference was due to a longer inverted terminal repetition (ITR) in P2 DNA. Both the P-1 and P-2 ITR genotypes remained unchanged after five additional virus passages. These findings indicate that infectious Ad18 genomes can carry ITRs of different length. Based on direct nucleotide sequence analysis, the P-1 ITR is 165 bases long and possesses extensive homology with the ITR of Ad12. In addition, the P-1 contains several short base tracts that are variably present in the ITRs of more distantly related serotypes. One of these tracts (5'...TGACGT) is also found near the ends of DNA from both autonomous and Ad-dependent (AAV) parvoviruses. It is clear that ITR sequences play fundamental roles in virus DNA replication and virus-cell interactions (e.g., oncogenesis).

Poxviruses

Structure and Function of Poxvirus Genomes

A. Ends of the vaccinia virus genome consist of hairpin loops.

The nature of the ends of the vaccinia virus genome was determined by nucleotide sequencing. Our finding of terminal hairpins indicated that the linear double-stranded DNA molecule consists of a single continuous polynucleotide chain. The 104 nucleotide apex of the hairpin contains predominantly A and T residues and is incompletely base-paired. These loops exist in two forms, which when inverted with respect to each other are complementary in sequence. Both forms of the 104 nucleotide loop are present in nearly equimolar amounts at each end of the genome. A set of 13 tandem 70 bp repeats begins 87 bp from the proximal segment of the terminal loop, followed by a unique sequence of 325 bp, and then by a second set of 18 tandem 70 bp repeats. The last of the 70 bp repeats is overlapped by the first of two tandem 125 bp repeats which are followed by eight tandem 54 bp repeats. Nucleotide sequence homologies suggest that the 74 and 125 bp repeats arose by unequal recombination between tandem 54 bp repeats or their precursors. Models for the replication of the terminal segment of the poxvirus genome that involve site-specific nicking and lead to flip-flop rearrangements have been proposed. Similar mechanisms may be involved in the synthesis of the eukaryotic chromosome.

B. Genetic evidence for a vaccinia virus encoded DNA polymerase.

Phosphonoacetate (PAA), at concentrations of 200 μg/ml or more, prevented growth of vaccinia virus in HeLa and BSC-1 cells. Spontaneous vaccinia mutants, selected at high PAA levels, were resistant to the anti-viral effects of the drug. The action of PAA was directed toward an early viral function since the drug was inhibitory only during the first 4 h of the approximately 15 h growth cycle. Conversely, significant reversal of the antiviral effects was obtained only when the drug was removed at or before the fourth hour of infection. Incorporation of [^{3}H]thymidine into cytoplasmic viral DNA was severely inhibited in cells infected with wild-type but not mutant virus. Virus-induced DNA polymerase isolated from the cytoplasm of cells infected with wild-type or mutant virus had indistinguishable chromatographic properties on DEAE- and phosphocellulose columns. However, the wild-type enzyme was inhibited by relatively low concentrations of PAA, whereas ten-fold higher concentrations were needed for equivalent inhibition of the mutant enzyme. Kinetic analysis indicated that PAA inhibition was non-competitive with deoxyribonucleoside triphosphates; Ki values for wild-type and mutant DNA polymerases were approximately 25 μM and 300 μM, respectively. Inhibition of wild-type polymerase was immediate and complete even when PAA was added after initiation of DNA synthesis *in vitro*, suggesting that chain elongation was affected. These results established that the DNA polymerase is a target of the antiviral action of PAA and provided genetic evidence that this enzyme is virus encoded.

Regulation of Vaccinia Virus Gene Expression

A. Nucleotide sequence of two early genes.

The proximal part of the 10,000 base pair (bp) inverted terminal repetition of vaccinia virus DNA encodes at least three early mRNAs. A 2,236 bp segment of the repetition was sequenced in order to characterize two of the genes. This task was facilitated by constructing a series of recombinants containing overlapping deletions; oligonucleotide linkers with synthetic restriction sites provided points for radioactive labeling prior to sequencing by the chemical degration method of Maxam and Gilbert. The ends of the transcripts were mapped by hybridizing labeled DNA fragments to early viral RNA and resolving nuclease S1 protected fragments in sequencing gels, by sequencing cDNA clones, and from the lengths of the RNAs. The nucleotide sequences for at least 60 bp upstream of both transcriptional initiation sites are more than 80% A T rich and contain long runs of As and Ts with some homology to prokaryotic and eukaryotic consensus sequences. The gene transcribed in the rightward direction encodes an RNA of approximately 530 nucleotides with a single open reading frame of 420 nucleotides. Preceding the first AUG, there is a heptanucleotide that can hybridize to the 3' end of 18s rRNA with only one mismatch. The derived amino acid sequence of the protein indicated a molecular weight of 15,500. The gene transcribed in the leftward direction encodes an RNA 1,000 to 1,100 nucleotides long with an open reading frame of 996 nucleotides and a leader squence of only 5 to 6 nucleotides. The derived amino acid sequence of this protein indicated a molecular weight of 38,500. The 3' ends of the two transcripts were located within 100 bp of each other. Although there are A T rich clusters near the putative transcriptional termination sites, specific A_2TA_3 poly(A) signal sequences are absent.

B. Colinearity of mRNAs with the Vaccinia Virus Genome: Absence of Splicing.

The colinearity of RNA transcripts with the vaccinia virus genome was investigated. Cytoplasmic RNA from infected cells was annealed to a cloned DNA segment that extended from 9 to 15.6 kilobase pairs from the left end of the genome and contained approximately 800 base pairs of the inverted terminal repetition (ITR). Remaining unhybridized single strands of DNA were digested with nuclease S1, and the lengths of the protected DNA fragments were determined by agarose gel electrophoresis under neutral and alkaline conditions. Uniformly ^{32}P-labeled cloned DNA insert, separated recombinant DNA strands, and smaller restriction fragments, as well as 3' and 5' end-labeled DNA, were employed to map five early RNAs and one late RNA. One of the early RNAs hybridized to sequences within the ITR, and the other four hybridized to sequences proximal to the ITR. The late RNA was initiated proximal to the ITR but extended into it. Interestingly, the 3' portion of this late RNA was complementary to the early RNA transcribed from the opposite strand of the ITR. From a comparison of the lengths of the protected DNA fragments on neutral and alkaline gels, all except the complementary early and late RNAs appeared to be colinear with the genome. Although the anomalous nuclease S1 data obtained with the latter RNAs mimicked splicing, they were shown by DNA-DNA hybridization to result from a small deletion or rearrangement within the ITR. Thus far, no true examples of spliced vaccinia virus RNAs have been found.

C. Mapping of the vaccinia virus thymidine kinase gene by marker rescue.

A selective plaque assay that uses thymidine kinase (TK)-deficient human 143 cells was developed to titer mixtures of TK^+ and TK^- vaccinia virus. With this assay, it could be shown that methotrexate-resistant TK^+ virus was formed in cells coinfected with TK^-virus and wild-type virus DNA. By substituting vaccinia DNA fragments cloned in plasmids for virion DNA, this marker rescue system provided the basis for mapping the TK gene. Of the 15 HindIII fragments, only J could rescue five independently derived TK^- mutants. This 5,000-base pair (bp) fragment maps approximately 80,000 bp from the left-end of the 180,000 bp vaccinia genome. Marker rescue could be detected with 18 ng or less of plasmid and was proportionate to DNA concentration. The resistance to methotrexate of the TK^+ recombinants was shown to be due to TK synthesis. Evidence that the HindIII J fragment contains the structural TK gene and not a regulatory element was demonstrated by the synthesis of active TK in a cell-free system programmed with mRNA selected by hybridization to the plasmid. Our previous studies indicated that mRNAs coding for three immediate early polypeptides with molecular weights of 41,000, 21,000, and 17,000 map within HindIII J. The mapping of the easily selectable vaccinia virus TK gene now opens the way to genetic manipulations that should increase our understanding of vaccinia virus gene expression and facilitate the use of vaccinia virus as an efficient cloning vector for foreign genes.

Vaccinia Virus: An Infectious Selectable Eukaryotic Cloning Vector

A. Inactivation of the thymidine kinase (TK) gene by site directed recombination in vivo.

The thymidine kinase gene was interrupted by ligating an *Eco*RI fragment of adenovirus DNA to the *Eco*RI cleaved *Hin*dIII J fragment of vaccinia virus cloned in pBR322. This recombinant was then used to transfect cells that had been infected with wild-type vaccinia virus. TK^- recombinants were selected by plaquing in the presence of BudR. Restriction endonuclease analysis and blot hybridization confirmed that *in vivo* recombination had occurred.

B. Expression of the herpes virus thymidine kinase gene fused to a vaccinia virus promoter.

A 250 base pair DNA fragment that includes the initiation and putative promoter sequences of an early vaccinia virus gene was fused to the body of the herpes virus TK gene. The hybrid DNA was then flanked by vaccinia virus DNA and then used to transfect cells that had been infected with TK^- vaccinia virus. TK^+ recombinants were selected by plaque assay in medium containing thymidine and methotrexate. Restriction endonuclease analysis and DNA hybridization identified herpes virus sequences in the recombinant virus. Additional studies demonstrated that the herpesvirus thymidine kinase was expressed.

Functional Analysis of Vaccinia Virus DNA

The production and ordering of unique segments of vaccinia virus DNA provides physical maps of the genome. The isolation of mutants of vaccinia virus can be used to develop a functional map of this genome. Mutants which are resistant to the drug aphidicolin, a known inhibitor of eukaryotic DNA polymerase α and of the DNA polymerase of herpes and vaccinia virus, can locate the region of the DNA polymerase gene. To develop an understanding of the effects of the drug, tests were made on the uptake of tritiated thymidine into virus infected and mock infected HeLa cells. The results indicate that the drug can be used to suppress cell synthesis at a concentration of 5 μM, so that the radioactivity incorporated into an infected cell reflects mostly viral DNA synthesis. The radioactivity from infected cells incubated with drug at 5 μM hybridizes almost excusively to vaccinia DNA, whereas control DNA from drug treated cells hybridizes only slightly to vaccinia DNA. This differential drug sensitivity provides a test for virus mutants which exhibit changed rates of DNA synthesis. A desirable experimental feature of this drug is that its effects on DNA synthesis are readily reversible.

Compared with controls, there is no reduction in the infectious titer of virus in the presence of drug at 20 μM. At 80 μM, the *apparent* titer is reduced by several logs. Estimates of the reduction in titer are complicated by the rapid decrease in plaque size which occurs over the range of 20 to 100 μM. Spontaneous mutants which form plaques of normal size are not found after a one step growth cycle in the presence of the drug. A preliminary test with a virus stock which had been mutagenized with hydroxylamine produced normal plaques when grown at 80 μM drug concentration, after a single cycle of growth. Repeated passage of virus stocks at increasing drug concentrations should amplify the population of spontaneous drug resistant virus.

Honors and Awards

Dr. Norman P. Salzman continued to serve on the Editorial Board of the Journal of Virology, The Editorial Advisory Board, Biochemistry, the Scientific Board of the Coordinating Council for Career Research, and the NIAID Promotion and Tenure Committee. He serves as Professorial Lecturer, Georgetown University School of Medicine and was Chairman of the Papovavirus Workshop at the first meeting of the American Society for Virology. He co-authored two book chapters, one with Roger Monier entitled "History and Overview of Oncogenic DNA Viruses" for inclusion in "Viruses Associated with Human Cancer" edited by Dr. Leo A. Phillips, published by Marcel Dekker and one with Michael Seidman entitled "*In vivo* Replication of SV40 DNA", for inclusion in "Molecular Events in the Replication of Viral and Cellular Genomes", published by Martinus Nijhoff.

Dr. Moss continues to serve on the editorial boards of the Journal of Virology and of Antimicrobial Agents and Chemotherapy and as an Associate Editor of Virology. This past year, he has been apointed to the Editorial Board of the Journal of Biological Chemistry and to the Molecular Biology Study Section of the American Cancer Society. Dr. Moss was elected Chairman of the 1983 Gordon Conference on Animal Cells and Viruses and was invited to prepare a review on capping of messenger RNA for the Annual Review of Biochemistry.

Dr. Lois Salzman coauthored a chapter entitled "Replication of Parvovirus DNA" in "Developments in Molecular Virology, Vol. 2 (Martinus Nijhoff and Dr. W. Junk, publishers).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00123-16 LBV

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Structure and Replication of Poxvirus Genomes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Bernard Moss	Medical Director	LBV, NIAID
Others:	Elaine Jones	Staff Fellow	LBV, NIAID
	Bahige Baroudy	Visiting Fellow	LBV, NIAID

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Biology of Viruses

SECTION
Macromolecular Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.6	1.6	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Poxviruses are large DNA viruses that replicate in the cytoplasm of infected cells. Efforts are being made to determine the structure and mode of replication of poxvirus DNA which in some respects resembles the eukaryotic chromosome. Vaccinia virus, the prototype for this group, has a 180,000 base pair (bp) linear double-stranded DNA genome with covalently linked ends and a 10,000 bp inverted terminal repetition. Nucleotide sequence analysis revealed that the ends of the genome consist of hairpin loops so that the two DNA strands form one continuous polynucleotide chain. The 104 nucleotide apex of the loop is A·T rich, incompletely base-paired and exists in two isomeric forms that are inverted and complementary in sequence (flip-flopped). The terminal loop is followed by several sets of tandemly repeating sequences. Models for the replication of the terminal segment of the poxvirus genome that involves site-specific nicking and lead to flip-flop rearrangements and head to head dimerization have been proposed. Genetic evidence that vaccinia virus encodes its own DNA polymerase has been obtained.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00124-13 LBV

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Replication of viral specific RNA in a rat nephroma cell infected with the parvovirus KRV

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	Lois A. Salzman	Research Chemist	LBV, NIAID
Others:	Rita Mitra	Visiting Fellow	LBV, NIAID

COOPERATING UNITS (if any)

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NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
	1.5	.3

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) We are following the production of the parvovirus, KRV, as a tool to understand both cellular and viral mRNA synthesis. Viral specific RNAs are synthesized in the nucleus, processed, modified and transported to the cytoplasm, presumably by the same mechanism as cellular mRNA biosynthesis. However, viral RNA synthesis involves fewer species of synthesized RNA than total cellular RNA. Hence, it is a simpler system for study than the complex eukaryotic cellular RNA. The viral RNAs can be identified as viral specific by hybridization, gel electrophoresis, restriction enzyme analysis and by Northern and Southern blotting techniques.

We have isolated and partially characterized at least five KRV specific RNAs in the infected rat nephroma cell. These RNAs are probably the functional messages. The longest KRV RNA is 4.7 Kb and would represent a transcript of 95 to 100% of the viral genome. The most abundant message, about 3.0 Kb, represents over 50% of the viral genome and probably codes in the reticulocyte transcribing system for the most abundant viral protein (mw 68,000) which is the main viral capsid protein. This abundant RNA, on the basis of R loop data, has an origin of transcription about 0.38 to 0.42 map units from the 3' end of the single stranded KRV genome.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00126-09 LBV

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Functional Analysis of Vaccinia Virus DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Frank M. DeFilippes	Research Physicist	LBV, NIAID

COOPERATING UNITS (if any)

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SECTION
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INSTITUTE AND LOCATION
NIAID, National Institutes of Health, Bethesda, MD 20205

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The drug aphidicolin inhibits the synthesis of DNA from vacccinia virus and HeLa cells. The primary effect of the drug is through interaction with DNA polymerase. Tests at times up to six hours after infection indicate that viral DNA synthesis (5 pfu/cell) is reduced to about 40, 24, 15, and 12% at drug concentrations of 5, 10, 15, and 20 μM compared with controls. The same comparison shows that cell DNA synthesis is reduced to 3, 2, 1 1/2, and 1% at these drug concentrations. At 1 μM drug, cell synthesis is about 6% of control values.

At 20 μM, aphidicolin does not reduce the titer of infectious virus at 56 hours after infection, but does produce small plaques. At 80 μM, the titer appears to be reduced by several logs. The drug is extremely reversible for both DNA synthesis and production of infectious virus.

Physical maps have been derived for phage 42, a bacterial virus with a circularly permuted DNA.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00290-01 LBV

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Site Specific and Deletion Mutants which Regulate SV40 Transcription

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P. I.	John Brady	Staff Fellow	LBV, NIAID
Others:	N. P. Salzman	Chief	LBV, NIAID

COOPERATING UNITS (if any)

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SECTION
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NIAID, National Institutes of Health, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL: 1.0	OTHER: .4

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In most eucaryotic genes, a common set of nucleotides has been found before the start site of RNA transcription. These nucleotides, referred to as either a TATA or Goldberg-Hogness box, are important determinants of transcription in vivo and in vitro. The control region for the late SV40 transcripts have been examined by site specific mutagenesis and by generation of deletion mutations. Mutations that enhance or suppress the transcriptional activity of a single start site have been identified and differ from the consensus Goldberg-Hogness box.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00291-01 LBV

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Interaction of RNA Polymerase II with Defined DNA Templates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Norman P. Salzman	Chief	LBV, NIAID
Other:	Helena Mishoe	Staff Fellow	LBV, NIAID
	Ami Vonsover	Visiting Fellow	LBV, NIAID
	John Brady	Staff Fellow	LBV, NIAID

COOPERATING UNITS (if any)

Dr. Gerald Lancz, Dept. of Medical Microbiology, University of South Florida, Tampa, Florida

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INSTITUTE AND LOCATION
National Institutes of Health, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
	2.6	1.3

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The interaction of purified calf thymus RNA polymerase II and SV40 or adenovirus DNA have been examined. The enzyme binds to the DNA at specific sequences, but these sequences are not identical to the 5' start sites used in vivo or in vitro in a system containing a crude cell free extract. The enzyme also binds to nicks in DNA and forms the transcript which is an RNA-DNA adduct. During chain growth, the enzyme pauses or terminates RNA chains at specific sites.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00292-01 LBV

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Protein Factors Required for Specific Transcription

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Norman P. Salzman	Chief	LBV, NIAID
Others:	R. Natarajan	Visiting Fellow	LBV, NIAID
	Gokul Das	Visiting Associate	LBV, NIAID

COOPERATING UNITS (if any)

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Laboratory of Biology of Viruses

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NIAID, National Institutes of Health, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
	2.3	1.3

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In vitro transcription systems are used to identify promoter signals and factors that control transcription in eucaryotic systems. These systems accurately initiate transcription from a wide variety of promoters. We find that for optimal transcription, proteins in the extracts bind to the DNA, and protect the DNA from micrococcal nuclease digestion. High DNA concentrations inhibit transcription, but precise transcription can be restored by the addition of the four histones H_2A, H_2B, H_3 and H_4.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 01293-01 LBV

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Characterization of proteins associated with the parvovirus, KRV, and its DNA.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Lois A. Salzman	Research Chemist	LBV, NIAID
Others:	Dale Brown	Guest Worker	LBV, NIAID
	Phyllis Fabisch	Microbiologist	LBV, NIAID

COOPERATING UNITS (if any)

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SECTION
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INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL: 1.5	OTHER: 1.3

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Intracellular DNA intermediates of the parvovirus, KRV, are usually found associated with proteins with a capsid-like structure forming nucleocapsids. We have examined the replicative intermediate DNA of KRV to determine the associated characteristics of the protein(s). Protein is found associated with the 5' termini of the KRV double-stranded replicative intermediate. The molecular weight of the protein determined by electrophoresis in acrylamide gels is about 68,000. The protein remains attached to the DNA after centrifugation in alkaline cesium chloride. The stability of the DNA-protein linkage in alkali is believed to be due to a covalent linkage. The terminal amino acid in the DNA-protein linkage was determined to be phosphoserine. The terminal nucleotide is thymidine or cytidine. We hope to further investigate the function of the protein. We also hope to determine by peptide mapping and amino acid sequencing the relationship, if any, to the KRV capsid proteins.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00294-01 LBV

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Structure and Function of Adenovirus DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	James A. Rose, M.D.	Section Head, LBV, NIAID
Other:	Claude F. Garon, Ph.D.	Research Microbiologist, RML, NIAID
	James W. Garrison	Bio Lab Tech, LBV, NIAID

COOPERATING UNITS (if any)
R. Padmanabhan, University of Kansas School of Medicine
Kansas City, KS 66103

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NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL: .3	OTHER: 1.2

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☐ (a) HUMAN SUBJECTS XX☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The current objective of these studies has been the application of physical and biochemical techniques to characterize the length and structure of inverted terminal repeats (ITR's) in the genomes of both oncogenic and nononcogenic human adenoviruses (Ad). Our ultimate goal is to define the roles of these sequences in regulating viral and cell growth (e.g., infectious yield and efficiency of cellular transformation). We have now described several important structural features of Ad ITRs (i.e., size, variations and homologies). Among methods used are gradient sedimentation, DNA cleavage with restriction endonucleases, gel electrophoresis, base sequence analysis and electron microscopy.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00295-01 LBV

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Helper Factors Required for Expression of the Adeno-Associated Virus Genome

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John Janik, M.D.	Medical Staff Fellow	LBV, NIAID
Other:	James Rose, M.D.	Section Head	LBV, NIAID
	Frank Koczot, Ph.D.	Research Biologist	LBV, NIAID

COOPERATING UNITS (if any)

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NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
	1.2	1.6

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The main objectives of this project are (i) to define where and how each required helper virus factor regulates expression of defective human parvovirus (AAV) genomes and (ii) to relate these findings to their respective roles in the replication of the helper viruses (adenoviruses, herpesviruses) themselves as well as to potentials for selective interference with viral infection. We previously mapped the adenovirus genes required for AAV replication and have recently found that two of these gene products, VAI RNA and the 72K DNA-binding protein, are required for translation of AAV mRNA. Among methods used are cleavage of DNA with restriction endonucleases, DNA cloning, gel electrophoresis, blot-hybridization analyses and DNA transfection of cells.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00296-01 LBV

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Characterization and Production of Parvovirus Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Richard McPherson, M.D.	Research Associate	LBV, NIAID
Other:	James Rose, M.D.	Section Head	LBV, NIAID

COOPERATING UNITS (if any)

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INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL: 1.2	OTHER: .3

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☐ (a) HUMAN SUBJECTS X☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The main objectives of these studies are (i) to identify and characterize all proteins that are specified by the defective human parvoviruses (AAV) and to determine similarities and differences with autonomous parvovirus proteins, (ii) to define the mechanism(s) by which the AAV proteins arise and (iii) to define specific functions of the AAV proteins. We have now found several AAV structural protein species previously not known to exist. Post-translational processing does not appear to account for production of any AAV structural proteins, although they share large proportions of sequences-in-common. The mechanism that regulates translation of AAV proteins is of fundamental interest and is now being investigated. Among methods used are affinity chromatography, gel electrophoresis, in vitro translation of viral RNA and protease digestion analysis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00297-01 LBV

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mechanism and Regulation of Adeno-Associated Virus DNA Replication

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	James Rose, M.D.	Section Head	LBV, NIAID
Other:	Er-Chung Wang, Ph.D.	Visiting Associate	LBV, NIAID
	Edwin Sebring, Ph.D.	Research Chemist	LBV, NIAID

COOPERATING UNITS (if any)

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NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
	2.3	.9

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The primary objective of this project is to define biochemical mechanisms involved in eukaryotic DNA synthesis. To approach this problem, we are investigating adeno-associated virus (AAV) and adenovirus DNA replication in in vitro systems. We have now shown that replicating forms of AAV DNA can be generated in vitro utilizing either endogenous or exogenously added templates and cellular polymerases. These studies also demonstrate that de novo initiation of DNA synthesis can occur in vitro. Partial fractionation of extracts has been accomplished, and two potent inhibitors of AAV DNA synthesis have been purified. Among methods used are differential centrifugation, ion exchange and affinity chomotography, gel electrophoresis and isoelectric focusing.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00298-01 LBV

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Vaccinia Virus: An Infectious, Selectable, Eukaryotic Cloning Vector

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Bernard Moss	Medical Director	LBV, NIAID
Others:	Michael Mackett	Visiting Fellow	LBV, NIAID
	Geoffrey Smith	Visiting Fellow	LBV, NIAID

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Biology of Viruses

SECTION
Macromolecular Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.3	2.3	1.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Vaccinia virus is a large DNA virus that replicates within the cytoplasm of eukaryotic cells. We have succeeded in using this virus as a eukaryotic cloning vector. The initial step was construction of plasmid recombinants containing foreign DNA flanked by vaccinia virus DNA sequences. The plasmid DNA was then introduced into cells infected with vaccinia virus where homologous recombination occurred. Selection of viral recombinants containing foreign DNA inserted into the vaccinia thymidine kinase gene was achieved by plaque assay in the presence of 5-bromodeoxyuridine. Alternatively, the herpes virus thymidine kinase gene was inserted into a non-essential region of the genome of thymidine kinase negative vaccinia virus mutants, and selection was achieved by plaque assay in the presence of methotrexate. Expression of the herpes virus gene was dependent on vaccinia virus regulatory sequences. Restriction endonuclease analysis, agarose gel electrophoresis, and hybridization to ^{32}P-labeled DNA was used to confirm that site-specific recombination had occurred. The distinctive enzymatic properties of the herpes virus thymidine kinase was used to confirm expression of this gene.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00 307-01 LBV

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Regulation of Vaccinia Virus Gene Expression

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Others:	Bernard Moss	Medical Director	LBV, NIAID
	Mary Haffey	Staff Fellow	LBV, NIAID
	Gyorgy Bajszar	Visiting Fellow	LBV, NIAID

COOPERATING UNITS (if any)

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LAB/BRANCH

Laboratory of Biology of Viruses

SECTION

Macromolecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3.7	PROFESSIONAL: 2.7	OTHER: 1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Detailed transcriptional maps and translational maps of selected portions of the vaccinia virus genome were constructed. More than 12 early transcripts were characterized and two early genes were sequenced. These studies revealed that early mRNAs are colinear with the genome, unspliced, and have multiple closely spaced 5' ends. The nucleotide sequences for about 60 base pairs upstream of transcriptional initiation sites are extremely A T rich and contain long runs of As and Ts with some homology to prokaryotic and eukaryotic consensus sequences. The eukaryotic A_2TA_3 poly(A) signal sequence was not found near the ends of the genes. Marker rescue techniques were developed to map functional vaccinia virus genes. In this manner, thymidine kinase was mapped to the HindIII J fragment located near the center of the DNA molecule.

LABORATORY OF CLINICAL INVESTIGATION
1982 ANNUAL REPORT
TABLE OF CONTENTS

-Z01-AI Project Number		Page
Summary		18-1
00043-17	Immunology and Chemotherapy of Systemic Mycoses--Bennett	18-9
00045-14	Studies on the Interaction of Antibody and Complement on the Production of Immune Damage--Frank	18-10
00047-13	Clinical Studies of Patients with Known or Suspected Parasitic Disease--Ottesen	18-11
00048-12	The Pathophysiology of Autoimmune Hemolytic Anemia--Frank	18-12
00057-09	Basic Studies on Pathogenic Fungi--Kwon-Chung	18-13
00058-08	The Pathogenesis and Chemotherapy of Herpes Virus Infections in Man--Straus	18-14
00154-07	Immunologic Neurophysiologic, biochemical and cellular events in immediate hypersensitivity--Kaliner	18-15
00155-07	Phagocytic Cell Function--Gallin	18-16
00189-03	Clinical and Biochemical Studies of Human Enteral Adenovirus Infections--Straus	18-17
00192-04	Studies on Immediate Hypersensitivity--Metcalfe	18-18
00249-01	The Pathogenesis, Diagnosis, and Treatment of Systemic Mast Cell Disorders--Metcalfe	18-19
00250-01	Clinical and Laboratory Studies on Inflammatory Disorders of the Gastrointestinal Tract--Metcalfe	18-20
00269-01	Phagocytic Cell Activation--Seligmann	18-21
00270-01	Tubulin Tyrosinolation in Normal and Abnormal Human Neutrophils--Nath	18-22

LCI Table of Contents continued

-Z01-AI Project Number		Page
00271-01	Purification and Characterization of the Complement Proteins and Fragments--Hammer	18-23
00272-01	Host Defense Against Pneumococcal Bacteremia--Brown	18-24
00273-01	The Role of Fibronectin in Opsonization and Phagocytosis--Brown	18-25
00275-01	The Complement Receptor and C3 Mediated Opsonization--Gaither	18-26
00276-01	Studies of the Membrane Attack Complex of Human Complement--Joiner	18-27
00277-01	Mechanism of Serum Resistance in Bacteria--Joiner	18-28
00278-01	Studies of the Late Components of the Complement Cascade--Hammer	18-29
00279-01	Studies on Mucus Glycoproteins--Kaliner	18-30

Summary of Program
Laboratory of Clinical Investigation
October 1, 1981 September 30, 1982

Michael M. Frank, M.D., Chief of Laboratory
and Clinical Director, NIAID

Introduction

1982 has been an excellent year for the Laboratory of Clinical Investigation. From the beginnings of its present general structure 14 years ago, the laboratory has shown enormous promise, with high expectations of its impact in many areas of clinical investigation. During this fourteen year period there has been an increasing impact of laboratory programs as junior investigators have become more senior, scientific programs have been developed, and an increasing number of these programs have come to fruition.

Basically, the laboratory has developed a tripartite program with major emphasis on host defense mechanisms. Within that general formulation, however, is a fair amount of latitude. Thus, the allergy program has now established itself as a leader in the general areas of clinical allergy, pathophysiology of allergic conditions, and mediator function. Broad programs in asthma, mastocytosis and food allergy now exist within that group. Similarly, the infectious disease group, though concentrated in the general area of host defense mechanism, is sufficiently broad to include the Clinical Virology Section with its wide ranging interests in the molecular biology of herpes viruses and anti-viral agents. Within this overall framework of host defense, we have major interests in the pathophysiology of infectious disease, the role of specific microorganisms in the pathogenesis of infection, and the development of new tests to detect infection. Our clinical immunology group has emphasized immunologic damage, and the pathophysiologic effect of immune complex function and mechanisms of host defense.

As discussed in the section on honors, members of the laboratory each year receive increasing recognition as major figures in current investigation in their areas. As this fiscal year ends we are anticipating the inclusion of a new group, the Mucosal Immunity Section. This is discussed in greater detail under Administrative Changes. However, the addition of this new group gives us needed additional expertise in the general area of lymphocyte immunobiology and brings to the laboratory a world leader in the general area of immunity across mucosal surfaces and the pathophysiologic consequences of abnormalities in the immune mechanism. Although there have been a number of other administrative changes in the laboratory during the year with several new additions to the laboratory and the departure of one member of the laboratory for clinical practice, the overall themes have changed little.

SUMMARY OF LABORATORY PROGRAMS

The Allergic Disease Section

The allergic Disease Section has a long standing interest in neurologic and physiologic control mechanisms operant in allergic asthma. This has led to studies of autonomic control in asthma, as well as studies of mediators that contribute to allergic disease. Highly productive has been a model of mucous secretion which has been established in the laboratory over the last several years (Shelhammer, Marom, Kaliner). It has been long recognized that excess mucous secretion contributes in a major way to the morbidity and mortality of asthma. The laboratory has established a model to examine mucous secretion by airways. It has shown that anaphylaxis of airways or nasal mucosa leads to mucous release and the production of 5-HETE, 12-HETE and 15-HETE. Each of these HETE's is a mucous secretagogue as are SRS-A, histamine cholinergic and -adrenergic agonists, several prostaglandins and cyclic GMP. Prostaglandin generating factor of analyphylaxis (PGFA) also generates both mucous and HETE formation and may contribute to mucous production seen in anaphylaxis. Phagocytosis of particles by macrophages leads to the elaboration of low molecular weight non lipid material which is a potent secretor of mucous secretion, which has been termed macrophage-mucous-secretagogue. This material is released by pulmonary macrophages as well as peripheral blood monocytes. In response to zymosan ingestion or staphlococcal protein A interaction, mucous secretion from nasal and airway mucosa is inhibited by both corticosteroids and tetracyclene. The combination of these agents acts in an additive fashion suggesting that this combination may be efficacious in therapeutic situations.

Additional studies of autonomic nervous system control in allergic states have included studies of rabbits (Halonen, Kaliner). It was shown that rabbits immunized in a manner leading to selective IgE production have cholinergic hyperreactivity as do patients, suggesting a link between the immunologic and neurophysiologic abnormalities. Some subjects with adrenergic hyporeactivity were found to have antibodies directed at their adrenergic receptors, possibly providing a mechanism for such a neurophysiologic disturbance. Methods were worked out in the laboratory and have continued to be elaborated for quantitating both plasma histamine and urinary histamines by extremely sensitive techniques. These methods have been used to study patients with systemic mastocytosis. (Metcalfe, Kaliner, Frieri). Fourteen patients with systemic mastocytosis have been studied. Four patients had urticaria pigmentosa and three had idiopathic recurrent anaphylaxis. Patients with systemic mastocytosis were found to have an abnormal pre-liprotein. They also had an increase incidence of heart disorders including mitral valve prolapse. Bone disease was reflected in abnormal bone scans, low hydroxyprolines, and elevated Gla protein. A double blind study comparing disodium chromoglycate vs. chlorpheniramine and cimetadine has been initiated (Adkins, Frier, Metcalfe). Studies of the GI tract have included demonstration of two types of mast cells within the GI mucosa (Metcalfe, Saavedra Delgado). The typical mast cell responds to compound 48/80 with degranulation. Atypical mast cells do not . It was shown that oral administration of ovalbumin to ovalbumin sensitized animals resulted in histamine release from gastrointestinal tissues while chronic administration of ovalbumin resulted in an increase in the atypical mast cell number. Specific IgE immunoassays have also been developed for food allergins (Metcalfe, Saavedra-Delgado, and Subba-Rao).

Finally, cultured fibroblasts have been shown to both phagocytose and degrade mast cell granules leading to an increased rate of enzyme secretion by the fibroblasts (Metcalfe, Atkins, Friedman, Subba-Rao). This may be very important in regulating a immediate hypersensitivity responses. Mast cell granules were also found to inhibit lectin induced blastogenesis by human lymphocytes (Frieri, Metcalfe). This effect persisted after histamine removal.

The inhibitory factor was shown to be heparin proteoglycan. Thus, mast cell granules can alter lymphocyte function.

Clinical Immunology Section

The Clinical Immunology Section is interested in the role of antibody in complement in the host defense process. Studies have been divided into three or four general areas. These include: 1) Purification of complement components--study of thier interaction and mechanism of action; 2) Study of complement receptors and their biological function; 3) Studies of antigen antibody complexes, their interaction with complement and the role of the reticuloendothelial system in their removal from the circulation; and 4) Study of bacteria and their interaction with serum components including complement, the role of complement in opsonization and the factors that lead to clearance from bacteria from the circulation.

The complement bacteria work has been spearheaded by Drs. Eric Brown and Keith Joiner. Dr. Brown's work has led to characterization of the interaction of complement components with pneumococci. Evidence has been obtained that C3 molecules deposited by antibodies to different parts of the pneumococcal surface interact differently with C3b receptors. Electronmicroscopic pictures have shown those molecules in different locations on the pneumococci. C3b molecules deposited by anticapsular antibody interact efficiently with C3b receptors of phagocytic cells. These are located on the pneumococcal capsule. C3b molecules deposited by alternative pathway activation or anticapsular antibody interact poorly with C3b receptors and are located on the pneumococcal cell wall. These differences in location of C3b have the major influence on the pathogenecity for pneumococci. Basically, those C3b molecules not available to interact with C3b receptors do not cause adequate opsonization. Thus, in these situations C3b coated bacteria will continue to be highly virulent.

Dr. Keith Joiner has studied the mechanisms by which specific pathogenic bacteria evade killing by serum complement. In studies of a complement resistant and complement sensitive Salmonella Minnisota strain, it was shown that the resistant strain totally consumed terminal complement components through C9, whereas the sensitive strain consumed only small amounts of the terminal components. Studies using purified radiolabelled complement components indicated that a complement membrane attack complex (C5b-9) formed on the surface of the serum resistant strain but did not insert into the hydrophobic portions of the outer membrane and, therefore, was shed from the bacterial surface. In contrast, on the sensitive organism, hydrophobic interactions with the outer membrane occurred when the bacterium was killed. Subsequent studies with rough and encapsulated serum resistant S. pneumoniae showed that both strains caused substantial consumption of terminal complement components from human serum. Relatively stable deposition of C3 and C5-9 on the bacterial cell wall, was also demonstrated. Thus, the peptidoglycan layer appears to inhibit the complement mediated lysis. Presumably this layer prevents penetration of the membrane attack complex into the inner bacterial lipid bilayer membrane.

Studies have proceeded on complement receptor function as well. Endogenous neutrophil elastase was shown to cleave cell-bound C3b and C3bi, fragments of C3 which play a major role in phagocytosis (Gaither, Hammer). Viable PMN incubated with sheep erythrocytes bearing the C3 cleavage product, C3bi, induce release of a portion of the C3 molecule from the surface of the red cells resulting in a loss of binding to C3bi receptors. Release was blocked with normal human serum, whereas serum from patients with 1 anti trypsin deficiency had little inhibitory activity. Thus, 1 antitrypsin inhibits this activity of an important polymorph protease. It was shown that Raji cells express complement receptor CR1, CR2 and CR3. However, Raji cell Cr1 had a low affinity compared to normal lymphocytes and Raji cells failed to bind C4b a ligand which binds to CR1 on normal cells (Gaither, Magrath, Hammer, Gadek). Studies of the biochemistry of the late components have also proceeded. It was shown that the interaction of purified C5 and C6 in a low pH environment leads to formation of a 5,6 complex composed of a cleaved C5 chain and C6. This 5,6 complex functions similarly to the C5b.6 complex formed during normal activativation, although the complex has certain properties which differ from that of the usual C5,6. The formation of such a complex may partially explain the phenomena of acid lysis of PNH erythrocytes (Hammer, Frank, Shin, and Hänsch).

Studies have begun on the formation and mechanism of action of the C5-9 complex which inserts into biologic membranes. A new high-yield purification procedure for SC5-9 was developed. The yield from this procedure was 6-10 fold higher than that previously reported. Polyclonal antibodies to the SC5-9 neoantigen were prepared in goats and the antibodies were characterized. These antibodies will be used to study the formation of the 5-9 complex, its function in clinical samples, and its function on the surface of bacterial membranes (Joiner, Hammer, Frank, Lawley, Shin). This study represents one aspect of the extensive studies in purification of functionally active components that will be available for clinical studies. A new method of purification of C1 inhibitor has been developed (Prograis) which should lead to functionally active protein. C5a has been purified and is now under active study (Renfer, Hammer, Lawley, Yancie). Another serum protein which is under active study is fibronectin (Pommier, Brown). Emphasis to this point has been placed on exploring the role of fibronectin in the binding and ingestion of opsonized particles by monocytes. Evidence has been obtained that fibronectin enhances the binding and ingestion of IgG coated erythrocytes as well as C3b coated erythrocytes. Interestingly fibronectin increases ingestion of antibody and complement coated erythrocytes more than it increases binding of these erythrocytes. It appears that fibronectin acts through a trypsin sensitive receptor on monocytes which is not present on polymorphonuclear leukocytes. Fluorescent activated cell sorting assays have been developed to quantitate functional activity of these receptors. ELISA assays have been developed for both guinea pig and human fibronectin with a sensitivity of less than thirty nanograms/ml. This assay is being used to measure fibronectin levels in patients with a variety of disorders (Brown and Pommier).

Studies of the pathophysiology of autoimmune hemolytic anemia have also continued. Particular emphasis has been placed on Fc receptors, their enumeration and mechanism of action. It has already been shown that patients with certain rheumatic diseases characterized by circulating immune complexes and tissue deposition of these complexes have defects in reticuloendothelial system Fc receptor function (Frank). It has also been shown that such defects may precede the development of autoimmunity and may be present in patients with HLA types which predispose those patients to the development of autoimmunity. The discovery of this defect has led to further studies of Fc receptor function. Initial studies of Fc function on monocytes of HLA/B8 positive and negative individuals by a rosette technique showed no difference between these two groups, even though the HLA/B8 positive individuals have a high incidence of

Fc receptor clearance defects (Frank and Inada). For that reason a test to od enumerate Fc receptors on peripheral blood monocytes was developed (Fries and Frank). This allows quantitation of average number and association constant of Fc receptors. Again, no difference between HLAB8 positive and negative individuals was found. However, it was shown that patients with autoimmune hemolytic anemia as well as other hemolytic states, have an increased number of Fc receptors and that this number tends to return toward normal on glucocorticoid therapy. Studies of Fc receptor number and association constant in other autoimmune diseases like systemic lupus erythamatosous, as well as in normal individuals receiving glucocorticoids are continuing.

Finally studies have proceeded with patients with hereditary angioedema, (Brickman, Frank) as well as patients with paroxysmal nocturnal hemoglobinuria (Frank, Young) and autoimmune hemolytic anemia (Frank, Fries, Brown). Particular emphasis has been paid to signs of autoimmune disease in patients with hereditary angioedema. We now have a series of over 20 patients with autoimmune disease including granulomatous bowel disorders, glomerulonephritis, thyroiditis, Grave's disease, rheumatoid arthritis, etc. Ulcerations of the GI tract have been found to be common in hereditary angioedema. Patients with PNH have been shown to have abnormally low numbers of B-Fue and C-Fue in their peripheral blood, partially explaining the aplastic state noted in these patients. We have found that patients with chronic granulomatous disease have a marked decrease in their number of C3b receptors (Gaither, Gallin).

The Bacterial Diseases Section

The Bacterial Diseases Section has continued its studies of phagocytic cell function and the mechanism of phagocytic cell action. One of the most important studies conducted by this group this year was in the area of biochemistry of human neutrophil microtubule metabolism (Nath, Gallin). It was shown that a chemoattractant stimulates tubulin tyrosinolation in normal neutrophils. Studies in neutrophils from patients with Chediak-Higashi syndrome and chronic granulmatous disease indicated greatly exaggerated response of the former and a markedly inhibited response in the latter. These studies led to the conclusion that there was a close association between cellular redox state and tubulin tyrosinolation. Studies with a plant alkaloid, taxol, showed that this material inhibits bacterial neutrophil function without inhibiting neutrophil receptor expression. The alkaloid promotes and stabilizes assembled microtubules preventing normal microtubule turnover. It may function in this way in the production of chemotactic and other cellular disorders. Studies of phagocytic activation by synthetic oligopeptides conducted using membrane potential sensitive probes have also continued. Studies of the two population of neutrophils previously described, have revealed heterogeneity of binding among neutrophils. Cells that respond to chemoattractant with membrane depolarization have more chemoattractant associated with them than cells which do not. Thus, receptor studies of neutrophil suspensions need to be refined to consider heterogeneity among neutrophils (Seligmann, Gallin, Fletcher, Chused). It was also found by these workers that histamine inhibits superoxide formation by the chemoattractant but not by the calcium ionophore or phorbol myristate acetate. Of great importance is the recent demonstration that neutrophils contain a pool of chemotactic receptors with a density of specific granules that are mobilized to the plasma membrane with cell activation. The data support the concept that specific granules are a reservoir of receptors (Fletcher, Gallin, Donabedian, Buescher). Incubation of monocytes from a patient with Job's Syndrome with Staphylococcus aureous was shown to release an inhibitor of

PMN chemotaxis. In other studies leavamisol was found to be inferior placebo in preventing infection in patients with Job's Syndrome.

The Clinical Virology Section

The Clinical Virology Section has recently shown that a new class of virus, enteral adenoviruses, comprise two related groups of viruses which cause gastroenteritis in infants (Takiff, Straus). The major accomplishment of this group in the past year was the development and reporting of a new method for growing these viruses. Numerous isolates from around the world have now been grown in the laboratory and characterized by restriction endonuclease analyses. Virus specific antisera have been prepared and used in an ELISA assay for rapid detection of antigen in stool specimens. Outbreaks of enteral adenal virus associated gastroenteritis have also been demonstrated. The group has also studied herpes virus infections in a wide variety of hosts and has played a leading role in the use of systemic acyclovir in the treatment of herpes simplex infections (Straus, Takiff, Seidlin). Also molecular studies continuing to allow for rapid diagnosis of varicella and zoster infections and, as probes to determine sites of human viral latency.

Clinical Mycology Section

Extensive further chemical characterization of aspergillus fumigatus galactomannan has continued. Studies of immunization of normal volunteers with cryptococcal neoformans polysaccharide have also proceeded, and there have been induction of peripheral blood lymphocytes which secrete anticryptococcal antibody. At seven days polyclonal stimulation by mitogens decreased antibody secretion but by 1014 days polyclonal stimulation of lymphocytes increased antibody secretion. Ketoconazole was shown to a potent therapeutic agent in blastomycosis and histoplasmosis and a new HPLC assay was developed with members of the pharmaceutical development section for this drug (Bennett, Henderson, Makinde, Friedman). Studies of the genetics of cryptococcus neoformans have also continued with the development of new media for separating subspecies and characterization of the polysaccharide surface of the various cryptococcal types. New methods have been developing for the handling of these various cryptococcal subspecies, and studies of their pathogenesis continue (Kwon-Chung, Rhodes, Bennett, Min). In pathophysiologic studies in C5 sufficient and C5 deficient mice it was shown that acute fatal cryptococcal pneumonia occurred in C5-deficient mice but not C5-sufficient mice. Chemotaxis of neutrophils and mononuclear cells in response to the infection was markedly greater in the C5-sufficient mice. The effect of C5 on localization of growth of cryptococci appeared to account for the differences in survival times in these two groups of animals.

Clinical Parasitology Section

Although having a major interest in Clinical Parasitology, it has only been with the opening of the ACRF that the Clinical Parasitology group has had a chance to interact with members of the LCI, with their laboratories on the 11th floor of the Clinical Center. Efforts are being made to develop immuno diagnostic techniques to identify specific antigens and antibodies so as to determine the type, stage and degree of infection for several infections, including shistosomiasis, filariasis, onchocerciasis, and strongyloidiasis. In some cases IgE antibody techniques have been used to detect a number of these diseases (Ottesen, Hussain, Kaushal, Francis). Studies are ongoing on the pathophysiology and treatment of onchocerciasis. Studies are underway on the efficacy of mebendazole as well as the mechanism of action of diethylcarbamazine. Field studies involve examination and treatment of patients with this disorder, and the collection of suitable samples for study.

HONORS AND AWARDS

There have been many awards and honors given to members of the Laboratory of Clinical Investigation during the last year. Dr. Frank was presented with the Distinguished Service Medal, the highest medal of the United States Public Health Service. The award reads "For studies clarifying the pathophysiology of immune hemolytic anemia, the development of successful treatment of Hereditary Angioedema and pioneering studies on the molecular basis of complement activation." Dr. Frank was invited to be editor of a new journal of complement research and serve on the editorial board of a new journal "Clinical Immunology Reviews." He continues as a member of the editorial board of the Journal of Clinical Investigation, Blood, Reviews in Infectious Disease and the Journal of Allergy and Clinical Immunology. Dr. Frank has been invited to Chair the only complement symposium at the International Immunology Meeting in Kyoto, Japan in the summer of 1983. Dr. Frank was appointed as a member of the Scientific Board of the Leonard Wood Leprosy Memorial.

Dr. Kaliner was named a Director of the American Board of Allergy and Immunology 1982 - 1988. He was elected Secretary of the American Thorasic Society Scientific Assembly on Allergy and Clinical Immunology and President - elect for 1983. He was named a member of the postgraduate education committee of the American Academy of Allergy and will be Chairman in 1982. He continues as an editor of the Journal of Clinical Immunology.

Dr. Bennett was a Divisional Lecturer of the Annual Meeting of the American Society for Microbiology and was elected to membership in the American Clinical and Climatological Society. He served as a Workshop Co-Chairman at the Second International Symposium on Infection in the Immunocompromised Host in Sterling, Scotland. He was appointed as a member of the American College of Physicians Infectious Disease Committee for the educational review of infectious disease (MKSAP IV). He was reappointed to the Publications Committee of the Infectious Disease Society of America.

Dr. Kwon-Chung was presented the 1982 award of the International Society for Human and Animal Micology (ISHAM) at its 8th Congress in February of 1982. She was elected a Vice President of ISHAM for 1982 - 1986 and was appointed Chairwoman of the Annual Lecture Committee of the Medical Micological Society of the Americas for 1982 and 1983.

Dr. Ottesen was appointed Chairman of the Steering Committee for Filariasis of the World Health Organization for 1982 to 1985. Dr. Neva was the Carl Hartford Visiting Professor at Washington University School of Medicine 1982 and Dr. Nash received the Commendation Medal of the United States Public Health Service in 1982.

Dr. Gallin continues as a member of the editorial board of the Journal of Clinical Immunology, the Journal of Immunology and Inflammation. He was one of the members of the organizing committee of the Second International Workshop on Infection in the Immunocompromised Host.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00043-17 LCI

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immunology and Chemotherapy of Systemic Mycoses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	A.A. Makinde	Guest Worker	LCI, NIAID
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LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Mycology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.4	2.4	2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1. Aspergillus fumigatus galactomannan (GM) was found to consist of a core $\alpha(1\rightarrow 2)$ mannan and $\beta(1\rightarrow 5)$ or $\beta(1\rightarrow 6)$ galactose side chains. Hapten inhibition studies found that rabbit antibody bound only the side chains. Rapid hepatic clearance of intravenously injected GM occurred because of mannose-receptor mediated endocytosis by Kupffer cells. The hepatocyte galactose-receptor failed to bind GM because galactose is in a furanose and not a pyranose configuration.

2. Normal volunteers immunized 7 days earlier with polysaccharide of type A Cryptococcus neoformans had peripheral blood B lymphocytes which spontaneously secreted anticryptococcal antibody. Polyclonal stimulation by mitogens decreased antibody secretion at this time but promoted secretion at 10-14 days postimmunization.

3. Ketoconazole, a new oral antifungal agent, was effective in blastomycosis and histoplasmosis. An improved HPLC assay correlated with bioassay up to serum concentrations of 10 μg/ml.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00045-14 LCI

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies on the Interaction of Antibody and Complement on the Production of Immune Damage

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael Frank	Head, Clinical Immunology Section and Chief, LCI	LCI/NIAID
Other:	Carl Hammer	Senior Investigator	"
	Thelma Gaither	Research Biologist	"
	Lois Renfer	Chemist	"
	Hattie Gresham	Med. Tech.	"
	Chaim Brickman	Clinical Associate	"
	Lawrence Prograis	Medical Staff Fellow	"
	Shinichi Inada	Visiting Fellow	"

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.2	3.2	2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies of hereditary angioedema continue. Patients have been studied for signs of other related illness or drug toxicity. We now have over twenty patients with autoimmune disease and hereditary angioedema (granulomatous bowel disease, glomerulonephritis, thyroiditis, Grave's disease, and Rheumatoid Arthritis, etc.). A large subgroup have developed a disorder not thought to be autoimmune. These patients have ulcerations of the upper GI tract. Efforts are underway to purify the C1 inhibitor, to study its activity and to purify abnormal C1 inhibitor from a number of patients with acquired angioedema.

Patients with PNH have been shown to have abnormally low numbers of B-FUE and C-FUE in their peripheral blood, explaining the aplastic state in these patients.

Laboratory studies have emphasized complement receptors. These are cellular receptors important in phagocytosis. Peripheral blood monocytes have a C3b receptor and a C3bi receptor but do not have a C3d receptor. We have found that on culture on glass or plastic the cells show rapid alteration in their surface receptors and develop a C3d receptor.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00047-13 LCI

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Clinical Studies of Patients with Known or Suspected Parasitic Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. A. Ottesen	Head, Clinical Parasitology	LCI/LPD,	NIAID
	T. E. Nash	Senior Investigator	LCI/LPD,	NIAID
	F. A. Neva	Senior Investigator & Head	LCI/LPD,	NIAID
Others:	M. Hofstetter	Research Associate	LPD,	NIAID
	H. Francis	Medical Staff Fellow	LPD,	NIAID
	M. Barral	Guest Worker	LPD,	NIAID
	A. Barral	Visiting Fellow	LPD,	NIAID
	R. Hussain	Sr. Staff Fellow	LPD,	NIAID
	N. Kaushal	Visiting Fellow	LPD,	NIAID
	A. Cheever	Asst. Chief	LPD.	NIAID
	T. Lawley	Medical Investigator	DB,	NCI
	P. Smith	Guest Worker	LMI,	NIAID

COOPERATING UNITS (if any) Inst. of Dermatology, Santa Domingo, Dominican Republic (Bogaert); Tuberculosis Res. Ctr (S.P. Tripathy) and Med. Col. of Madras (K.V. Thiruvengadam), Madras, India; San Juan Lab., CDC, (E. Ruiz-Tiben); Onchocerciasis Chemotherapeutic Res. Ctr., Tamale, Ghana (K. Awadzi).

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Parasitology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

IgE antibodies appear important in protective immunity to helminths. Their evaluation in patients with filariasis, onchocerciasis, schistosomiasis and strongyloidiasis indicates that variations in individual responses reflect differences in regulation of antibody production rather than differences in recognition of immunogens. Clinical studies have defined a state of diminished immune responsiveness to parasite antigens in these helminth infections and in leishmaniasis and trypanosomiasis (Chagas). The mechanisms responsible for cellular immune suppression include serum factors, adherent mononuclear cells and histamine while those responsible for limiting immediate hypersensitivity responses involve specific IgG blocking antibodies. After chemotherapy these suppressor mechanisms markedly diminish or dissappear.

Immunodiagnostic studies have identified specific antigens and antibody systems able to distinguish the type, stage and degree of infection for several infections. Strain differences defined among the protozool parasites, leishmania and giardia, are important in determining both the character of the host response evoked in infected individuals and their response to treatment.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 A1 00048-12 LCI

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Pathophysiology of Autoimmune Hemolytic Anemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael Frank	Head, Clinical Immunology Section and Chief, LCI	LCI/NIAID
Others:	Louis Fries	Clinical Associate	LCI/NIAID
	Shinichi Inada	Visiting Fellow	"
	Eric Brown	Senior Investigator	LCI/NIAID

COOPERATING UNITS (if any)
Thomas Lawley (DB/NCI) and G. Crabtree (LP/NCI)

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies continue on the role of the reticuloendothelial system in the clearance of immune complexes and in the production of autoimmune disease. Particular emphasis has been placed on Fc receptors, their ennumeration, and mechanism of action. Patients with HLA B8 DRw3, earlier shown to have an Fc receptor specific clearance defect by in vivo studies of red cell clearance, have the normal number of Fc receptors on their peripheral blood mononuclear cells. This was shown in studies of rosette formation with both human and rabbit antibody coated red cells and in studies of specific Fc receptor number and affinity using monoclonal human proteins. Thus, the defect in Fc receptor specific clearance cannot be related to a defect in the peripheral blood monocyte at this time. Importantly, a large group of patients with autoimmune hemolytic anemia have been shown to have a marked increase in their cell membrane Fc receptor number with a normal association constant of their receptor protein. On steroid therapy, the receptor number decreases toward normal. The role of glucocorticoids in receptor number and clearance rates is being studied in normal volunteers. Preliminary data suggests that administration of glucocorticoids to normals down regulates Fc receptor number on peripheral blood monocytes.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00057-09 LCI

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Basic Studies on Pathogenic Fungi

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K.J. Kwon-Chung	Senior Investigator	LCI	NIAID
OTHER:	Judith C. Rhodes	Postdoctoral Fellow	"	"
	Kyung H. Min	Visiting Fellow	"	"
	John E. Bennett	Head, Clinical Mycology Section	"	"

COOPERATING UNITS (if any)

A.K. Bhattacharjee (NIAMDD)

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Laboratory of Clinical Investigation

SECTION
Clinical Mycology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	3	2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Topics of current studies include: 1) chemical structure of polysaccharide from AD serotype of Cryptococcus neoformans, 2) developing a new method for the formation and regeneration of protoplast in C. neoformans, and 3) developing a new medium for the separation of C. neoformans and C. neoformans var. gattii. Chemical study showed that the capsular polysaccharide from serotype AD isolates of C. neoformans contains two major polysaccharides. Structural characterization is under progress. Protoplast study revealed that unlike other yeasts, gluselase is ineffective in digesting the cell wall of C. neoformans. However, Mutanase was found to be effective in preparing the protoplasts. Over 90% of C. neoformans cells became protoplast within 15 minutes exposure to the enzyme. At least 60% of the protoplasts regenerated when a new regeneration medium was used. The DNA obtained from the protoplasts was much less fragmented compared to the protoplasts obtained by other methods. Agar medium containing glycine, canavanine and bromthymol blue permitted the separation of C. neoformans var. gatti from C. neoformans within 5 days.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00058-08 LCI

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The pathogenesis and chemotherapy of herpesvirus infections in man.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. E. Straus	Senior Investigator, LCI, NIAID
Other:	H. Takiff	Medical Staff Associate, LCI, NIAID
	M. Seidlin	Medical Staff Associate, LCI, NIAID

COOPERATING UNITS (if any)

G. Vande Woude (LMV, NCI), J. Hay (USUHS), W. Ruyechan (USUHS), and R. Whitley (Birminhgam, Alabama).

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Medical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.5	PROFESSIONAL: 1.0	OTHER: 1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The pathogenesis, molecular biology, immunology, natural history, and therapy of human herpesvirus infections are being investigated. Immunocompetent and immunodeficient patients with a wide range of herpesvirus infections have been identified, followed clinically, diagnosed by virus isolation, and studied immunologically. During the past year we have made major advances in the treatment of selected patients with herpes simplex infections using a new drug, acyclovir.

Our major focus on the study of the molecular biology and latency of varicella zoster virus DNA has continued. During the past year we have completed restriction endonuclease analyses and molecular epidemiologic studies of 19 strains of varicella zoster virus DNA. We have molecularly cloned a library of varicella zoster DNA fragments and have completed the mapping of the varicella zoster virus genome. We are now using these recombinant clones for rapid diagnosis of varicella and zoster infections and as probes of human tissues to determine the sites and nature of viral latency.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00154 07 LCI

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Immunologic, neurophysiologic, biochemical and cellular events in immediate hypersensitivity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael Kaliner	Senior Investigator	LCI, NIAID
OTHERS:	Robert Lemanske	Staff Fellow	LCI, NIAID
	Thomas Casale	Staff Fellow	LCI, NIAID
	Marilyn Halonen	Guest Worker	LCI, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Allergic Diseases Section

INSTITUTE AND LOCATION
NIAID/NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Atopic patients have an array of autonomic abnormalities including cholinergic hyperresponsivity and β-adrenergic hyporeactivity. Rabbits immunized in a manner leading to selective IgE production were found to concomitantly develop cholinergic hyperreactivity suggesting a link between the immunologic and neurophysiologic abnormalities. Subjects with β-adrenergic hyporeactivity (atopic and others) were found to have antibodies directed at their β-adrenergic receptors possibly providing a mechanism for the neurophysiologic disturbance. Peripheral blood PMN and lymphocytes were found to lack demonstrable α-adrenergic receptors, but PMN do have histamine H-1 receptors. During the course of these studies, a technique for the rapid isolation of platelet-free lymphocytes from blood was developed. Plasma histamine (normal) = 318 ± 25 pg/ml and patients with systemic mastocytosis have marked elevations. Late phase allergic reactions were found to be dependent on early PMN infiltration, independent of complement and inhibited by combinations of H-1 and H-2 antihistamines.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 A100155-07 LCI

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Phagocytic Cell Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	John I. Gallin	Head, Bacterial Diseases Section	LCI,NIAID
Other:	Mark Fletcher	Expert	LCI, NIAID
	Haig Donabedian	Clinical Associate	LCI, NIAID
	E. Stephen Buescher	Clinical Associate	LCI, NIAID
	Deborah Cotton	Clinical Associate	LCI, NIAID
	Nancy Mounessa	Technician	LCI, NIAID
	Kerstin Cehrs	Technician	LCI, NIAID

COOPERATING UNITS (if any) Dr. D. Alling (NIAID, NIH), Dr. M. Friedman (Dept. Microbio. Georgetown Med. Ctr.), Dr. E. Schiffmann (NIDR, NIH), Dr. S. Hoffstein (Dept. Med., N.Y. Univ. Med. Ctr.), Dr. J. A. Charon (NIDR, NIAID).

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Laboratory of Clinical Investigation

SECTION

Bacterial Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.9	4.1	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The modulation of neutrophil (PMN) function has been studied by assessing chemoattractant receptors, cytoskeleton function, chemotaxis, degranulation and oxidative metabolism in normal and abnormal cells. The data indicate there is an intracellular pool of receptors for the chemoattractant fmet-leu-phe with the density of specific granules that are mobilized to the plasma membrane with cell activation. These data, together with studies in a patient with specific granule deficiency, support the concept that specific granules are a reservoir of receptors.

In studies of factors regulating chemotaxis abnormal "turn-off" of acute inflammatory reactions was seen in chronic granulomatous disease and a select defect in PMN superoxide generation was seen in pregnancy. Incubation of monocytes from patients with Job's syndrome with Staphylococcus aureus was shown to release an inhibitor of PMN chemotaxis. In other studies levamisole was found inferior to placebo in preventing infections in patients with Job's syndrome. New approaches for pharmacologic manipulation of phagocyte function were explored using Vitamin K. Vitamin K inhibited chemotaxis and oxidative metabolism without altering chemoattractant, Fc, or C3b receptor expression. The possibility Vit K or closely related compounds may be useful is currently under investigation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00189-03 LCI

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Clinical and Biochemical Studies of Human Enteral Adenovirus Infections.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Stephen E. Straus	Senior Investigator, Medical Virology Section, LCI, NIAID
Other:	H. Takiff	Medical Staff Fellow, Medical Virology Section, LCI, NIAID

COOPERATING UNITS (if any)

A.Z. Kapikian (LID, NIAID), H.S. Ginsberg (Columbia Univ.), J. Rose (LBV, NIAID), R. Yolken (Johns Hopkins), J. Esparza (Caracas, Venezuela).

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Medical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Enteral adenoviruses (EAds) comprise two related groups of viruses which appear to cause gastroenteritis in infants. Studies of these viruses and the diseases they produce were hampered until recently by an inability to grow them in tissue culture. During the past year we have developed and reported an already widely used method for growing these viruses. Numerous isolates from around the world have now been grown in our laboratory and are characterized by restriction endonuclease analyses. These analyses have permitted us to distinguish epidemiologically unrelated strains from around the world. Virus-specific antisera have been prepared and used in an ELISA system for rapid detection of antigen in stool specimens. EAd DNA fragments have been cloned in plasmid vectors, and the first DNA restriction maps have been completed. We have also completed collaborative studies documenting an outbreak of EAd-associated gastroenteritis in which we showed that respiratory illness is also a common feature of EAd infection. Our current efforts are directed at better defining the structures of the viral genomes and the homology between the EAd groups and conventional adenoviruses.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00192-04 LCI

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies on Immediate Hypersensitivity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.D. Metcalfe	Senior Clinical Investigator	LCI NIAID
Other:	F.M. Atkins	Senior Staff Fellow	LCI NIAID
	M. Frieri	Clinical Associate	LCI NIAID
	P.V. Subba-Aao	Visiting Scientist	LCI NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Allergic Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 3.5	PROFESSIONAL: 2.5	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Cultured fibroblasts have been shown to both phagocytose and degrade mast cell granules. This phagocytis process is both time and temperature dependent, and results in an increased rate of enzyme secretion from fibroblasts. This ability of fibroblasts to remove mast cell granules may represent an important mechanism by which immediate hypersensitivity reactions are terminated.

In studies using human lymphocytes, mast cell granules were found to inhibit lectin-induced blastogenesis. This effect persisted after histamine removal. The inhibitory factor was shown to be heparin proteoglycan. Thus, mast cell granules, by virtue of their heparin content, can alter lymphocyte function.

Human basophils were found to contain a chondroitin sulfate proteoglycan rather than heparin. This chondrontin sulfate could be released using calcium ionophore. Functional capabilities examined included anticoagulent activity and histamine-binding capacity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00249-01 LCI

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
The Pathogenesis, Diagnosis, and Treatment of Systemic Mast Cell Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.D. Metcalfe	Senior Clinical Investigator	LCI, NIAID
OTHERS:	M. Kaliner	Senior Clinical Investigator	LCI, NIAID
	M. Frieri	Clinical Associate	LCI, NIAID

COOPERATING UNITS (if any)

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Laboratory of Clinical Investigation

SECTION
Allergic Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Fourteen patients have now been examined for systemic mastocytosis. Seven of these patients were found to have systemic mastocytosis, four had urticaria pigmentosa and three were diagnosed as having idiopathic recurrent anaphylaxis. Those with systemic mastocytosis were found to have an abnormal pre-beta lipoprotein. Also found was an increased incidence of cardiac disorders including mitral value prolapse. Bone disease was reflected in abnormal bone scans low hydroxyprolines, and elevated Gla protein. Six patients with systemic mastocytosis are entered into a double-blind single crossover study comparing the therapeutic benefit of cromolyn sodium vs. chlorpheniramine and cimetadine.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00250-01 LCI

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Clinical and Laboratory Studies on Inflammatory Disorders of the Gastrointestinal Tract

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.D. Metcalfe	Senior Clinical Investigator	LCI NIAID
Other:	A. Saavedra-Delgado	Medical Officer	LCI NIAID
	P.V. Subba-Rao	Visiting Scientist	LCI NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Allergic Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 1.3	PROFESSIONAL: 1.3	OTHER:

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Typical and atypical gastrointestinal mast cells have been enumerated throughout the rat gastrointestinal system and correlated with tissue histamine. Typical mast cells degranulated after parenteral administration of compound 48/80, while atypical mast cells did not. These findings support the concept of heterogeneity in gastrointestinal mast cells. Acute administration of ovalbumin (OA) to OA-sensitized animals resulted in histamine release from gastrointestinal tissues, while chronic administration of OA resulted in an increase in atypical mast cell number.

A sensitive enzyme-linked immuno-assay (ELISA) to measure total and specific IgE has been developed. This assay is rapid, uses widely available reagents, and may be applied to the isolation of specific food antigens.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00269-01 LCI

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Phagocytic Cell Activation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	Bruce E. Seligmann	Staff Fellow	LCI, NIAID
	John I. Gallin	Head, Bacterial Diseases Section	LCI, NIAID
Other:	Mark P. Fletcher	Expert	LCI, NIAID
	Nancy Mounessa	Technician	LCI, NIAID

COOPERATING UNITS (if any) Dr. T. Chusid (LMI, NIAID).

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Bacterial Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 2.1	PROFESSIONAL: 1.6	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Modulation of phagocyte activation by the synthetic oligopeptide fmet-leu-phe has been studied using membrane potential sensitive fluorescent probes, assays of chemotaxis, and chemoattractant receptors, superoxide generation and degranulation in neutrophils from normal subjects, patients with select defects of phagocyte function and the use of certain pharmacologic probes. The data indicate modulation of fmet-leu-phe receptor affinity, through either negative cooperativity or changing populations of heterogenous receptors may be an important mechanism by which neutrophils adapt and respond to a gradient of chemoattractant during the process of chemotaxis. Related studies of two populations of neutrophils we described previously have revealed heterogeneity of fmet-leu-phe binding among neutrophils. Cells that respond to fmet-leu-phe with a membrane depolarization have more chemoattractant associated with them than cells which either do not respond or show a hyperpolarization. Thus, receptor studies of neutrophil suspensions need to be refined to consider heterogeneity among neutrophils. In other studies histamine was found to inhibit superoxide production by fmet-leu-phe but not by calcium ionophore A23187 or phorbol myristate acetate. This inhibition was prevented by cimetidine. Thus, pharmacologic manipulation of receptor function is possible in the future.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00270-01 LCI

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Tubulin Tyrosinolation in Normal and Abnormal Human Neutrophils

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	Jayasree Nath	Expert	LCI, NIAID
	John I. Gallin	Head, Bacterial Diseases Section	LCI, NIAID
Other:	Robert Roberts	Clinical Associate	LCI, NIAID
	Kerstin Cehrs	Technician	LCI, NIAID

COOPERATING UNITS (if any) Dr. M. Flavin (LCB, NHLBI, NIH).

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Bacterial Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.6	2.3	0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The biochemistry of human neutrophil (PMN) microtubule metabolism was studied by monitoring the specific stimulation of the post-translational incorporation of tyrosine into tubulin α-chains by the chemoattractant fmet-leu-phe. The data indicate fmet-leu-phe stimulates tubulin tyrosinolation in normal PMN. Studies in PMN from patients with the Chediak-Higashi syndrome and with chronic granulomatous disease indicated a greatly exaggerated response in the former and inhibited response in the latter. Based on studies of the oxidative state of the patients' cells and studies using oxidizing and reducing agents it is concluded that there is a close association between cellular redox state and tubulin tyrosinolation.

In related studies the plant alkaloid taxol was shown to inhibit neutrophil spreading, chemotaxis and bacterial activity without inhibiting expression of fmet-leu-phe, C3b or Fc receptors. Taxol promoted and stabilized assembled microtubules and caused microtubule bundle formation as well as inhibited tubulin tyrosinolation. The data provide new insights into the relationship between microtubule metabolism and neutrophil function.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00271-01 LCI

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Purification and Characterization of the Serum Complement Proteins and Fragments

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Carl H. Hammer	Senior Investigator	LCI/NIAID
Others:	Michael M. Frank	Clinical Director	"
	Keith Joiner	Senior Investigator	"
	Thomas Lawley	Senior Investigator	DB/NCI
	Kim Yancey	Medical Staff Fellow	"

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.45	PROFESSIONAL: 0.45	OTHER: 1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Alterations to our protocol for isolation of fully active and highly purified complement components have been incorporated to allow improved resolution and recovery of C3, C4, C5 and C9. A second stage DEAE-Sephacel column is used in which certain components (P, C3bINA, C2, C7, FB, C6 and C8) obtained now as complex pools from the initial column are rechromatographed under more favorable conditions. This step should generate components more amenable to further purification. C1 esterase inhibitor and C9 obtained from our protocol have been further purified. Polyclonal antibodies of high titre monospecific for many components have been produced in goats. We also have clones that produce monoclonal antibodies to complement components C3 and C3 fragments and also C9. The membrane attack complex (C5b-9) has been prepared utilizing a novel four step procedure. Antibody to the complex is being produced in a goat. Reactivity with neo-antigenic determinants on C5b-9 will be determined by Elisa assay. Trypsin digestion of C3 and subsequent sieve chromatography has generated C3a with vasoactive activity when tested in guinea pigs. C5 has been cleaved with Zx. Generation of C5a was evidenced by isolation of a 15,000 dalton bio-active fragment. When injected into normal human skin typical wheal and erythema reaction was seen.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00272-01 LCI

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Host Defense Against Pneumococcal Bacteremia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Eric Brown	Senior Investigator	LCI/NIAID
Others:	Keith Joiner	Senior Investigator	"
	Michael Frank	Clinical Director	"
	Carl Hammer	Senior Investigator	"
	Thelma Gaither	Biologist	"

COOPERATING UNITS (if any)

Melvin Berger, WRAMC

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TOTAL MANYEARS: 1.95	PROFESSIONAL: 0.95	OTHER: 1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Emphasis has been on characterizing the interactions of complement components with pneumococci. Evidence has been obtained that C3 molecules deposited by antibodies to different parts of the pneumococci interact differently with C3b receptors. Electron microscopic pictures have shown these molecules to be in different locations on the pneumococci. C3b molecules deposited by anticapsular antibody interact efficiently with C3b receptors and are located on the pneumococcal capsule. C3b molecules deposited by alternative pathway and/or anticapsular antibody interact poorly with C3b receptors and are located on the pneumococcal cell wall. The location of C3 deposition also affects the interaction of C3b with Factor B and Factor H. Studies of C5b-9 deposition on pneumococci and elution of MAC from the organisms have shown that these molecules insert into the organisms via hydrophobic interactions.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00273-01 LCI

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Role of Fibronectin in Opsonization and Phagocytosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Eric Brown	Senior Investigator	LCI/NIAID
Others:	Christine Pommier	Fogarty Fellow	"
	Michael Frank	Clinical Director	"

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NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Emphasis to this point has been placed on exploring the role of fibronectin in the binding and ingestion of opsonized particles by monocytes. Evidence has been obtained that fibronectin enhances the binding and ingestion of IgG coated erythrocytes and of C3b coated erythrocytes.

The augmentation of ingestion by fibronectin is more striking than its augmentation of binding. The fibronectin acts directly on monocytes, rather than interacting with the IgG or complement coated particle. Fibronectin acts through a trypsin-sensitive receptor on monocytes. Polymorphonuclear leukocytes and lymphocytes do not have this receptor.

ELISAs for both guinea pig and human fibronectin have been developed with a sensitivity of less than 30 ng/ml. These assays have been used to measure fibronectin levels in patients with systemic mastocytosis, cold urticaria, and hereditary angioedema.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00275-01 LCI

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Complement Receptor and C3 Mediated Opsonization

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thelma Gaither	Research Biologist	LCI/NIAID
Others:	Michael M. Frank	Clinical Director	"
	Carl H. Hammer	Senior Investigator	"
	James Gadek	Senior Investigator	IR PB/NHLBI
	Ian Magrath	Visiting Scientist	POB/COP/DCT/NCI

COOPERATING UNITS (if any)

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Endogenous neutrophil elastase was shown to cleave cell-bound C3b and C3bi, two fragments of C3 which play a major role in phagocytosis. When viable PMN were incubated with sheep cells bearing ^{125}I-C3bi, release of radiolabel occurred immediately, resulting in release of red cells from the PMN. C3b cleavage was slower and EC3b remained bound up to 30 min. Release was blocked with normal human serum whereas serum from patients with α 1 antitrypsin deficiency had little inhibitory effect. This suggests a mechanism for the in vivo regulation of PMN proteases. The expression of Complement Receptors (CR) by Raji cells and other Burkitt's lymphoma cell lines was studied in rosette assays and in competitive inhibition assays with purified C3 and C4 fragments. Raji cells expressed the three described C3 receptors, CR_1, CR_2, and CR_3. However, the affinity of Raji cell CR_1 was very low when compared with normal B lymphocytes and Raji Cells failed to bind to C4b, aligand which binds to CR_1 on normal cells. The significance of this difference with regard to receptor-mediated cell function is now under study.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00276-01 LCI

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies of the Membrane Attack Complex of Human Complement

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Keith Joiner	Senior Investigator	LCI/NIAID
Others:	Carl Hammer	Senior Investigator	"
	Michael Frank	Clinical Director	"
	Thomas Lawley	Senior Investigator	DB/NCI

COOPERATING UNITS (if any)
Dr. Moon Shin, University of Maryland

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INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.25	PROFESSIONAL: 0.25	OTHER: 1.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The membrane attack complex of complement causes much of complement mediated tissue damage. Its production is associated with the formation of neoantigens which can be used to identify it. A new high yield purification procedure for SC5b-9 was developed. Fresh human serum was activated with zymosan. The SC5b-9 complex was purified by precipitation with polyethylene glycol, affinity purification on anti-C5 sepharose and molecular seive chromatography. The yield from this procedure was 6-10 fold higher than previously reported. Polyclonal antibodies to SC5b-9 neoantigens were prepared in goats. Generation and characterization of monoclonal antibodies to these neoantigens is currently in progress. In the second phase of this project, high yield purification of C9 was accomplished by DEAE and hydroxylapatite ion-exchange chromatography and anticontaminant affinity column chromatography. Hydrophobic and hydrophilic fragments of C9 will be produced by α-thrombin cleavage, and monoclonal and polyclonal antibodies to these fragments will be produced. The antibodies to SC5b-9 neoantigens and C9 fragments will be used to study C5b-9 deposition in human tissues from patients with certain auto-immune disorders. C5b-9 orientation and localization in mammalian and bacterial membranes will also be studied.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00277-01 LCI

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mechanism of Serum Resistance in Bacteria

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Keith Joiner	Senior Investigator	LCI/NIAID
Others:	Eric Brown	Senior Investigator	LCI/NIAID
	Carl Hammer	Senior Investigator	"
	Keith Warren	Medical Technologist	"
	Michael Frank	Clinical Director	"

COOPERATING UNITS (if any)
Robert Goldman and Loretta Leive, LBP, NIADDKD; John Swanson, RML, LMSF, NIAID; Robert Dourmashkin, Mount Sinai School of Medicine, N.Y., N.Y.

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Laboratory of Clinical Investigation

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Clinical Immunology Section

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.95	0.95	1.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The mechanism by which specific pathogenic bacteria evades killing by the serum complement system has been studied. Initial studies with a serum resistant strain of Salmonella minnesota showed that the organism totally consumed terminal complement components through C9 whereas a serum sensitive mutant of the above parent strain consumed only small amounts of the terminal components. Studies using purified, radiolabelled terminal components indicated that a complement membrane attack complex (C5b-9) formed on the surface of the serum resistant strain. However the C5b-9 complex was shed from the surface and was not bactericidal because it did not insert into hydrophobic portions of the outer membrane. In contrast, the C5b-9 complex was inserted by stable, hydrophobic interactions into the outer membrane of the serum sensitive strain. Subsequent studies with rough and encapsulated serum resistant S. pneumoniae showed that both strains caused substantial consumption of terminal complement components from human serum. Relatively stable deposition of C5b-9 on the bacterial cell wall was demonstrated for both strains. Therefore, in serum resistant S. pneumoniae, there is an apparent failure of the attached complex to penetrate the thick peptidoglycan layer.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00278-01 LCI

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Studies of the Late Components of the Complement Cascade

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Carl H. Hammer	Senior Investigator	LCI/NIAID
Others:	Michael M. Frank	Clinical Director	"

COOPERATING UNITS (if any)
Moon Shin and Gertrude Hänsch, Dept. of Pathology, University of Maryland

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
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INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.4	OTHER: 0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
Complement mediated lysis of erythrocytes (E) is mediated by C5-C9, inserting into lipid bilayers of cell membranes. This requires binding and activation of C5 either by the classical or alternative pathway C5 convertases. We reported another mode of C5 activation which involves acidification to pH 6.4 of a mixture of C5 and C6. In the presence of C7, C8 and C9, the neutralized mixture, designated $C(56)^a$ generates a lytic activity for chicken E. We have performed a comparative study of the biological behavior of $C(56)^a$ and C5b,6. It was found that $C(56)^a$ is similar to C5b,6 in several properties. We have extended these observations on the functional behavior of $C(56)^a$ and described some of the complexes physico-chemical properties. The acid generation of $C(56)^a$ is dose dependent with both C5 and C6 and is almost as efficient as classical or alternative pathway activation. While $C(56)^a$ decays in the presence of C7 like C5b,6, the acid activated complex, in contrast to C5b,6 is also labile at 37°C in the absence of C7. Upon ultracentrifugation, acid activated C5 and C6 sediment consistent with the formation of a bimolecular 11S complex that coincides with the appearance of lytic activity. Analysis of the chain structure of acid treated C5 obtained from Egp ghosts, demonstrates a C6 dependent, C5 chain cleavage.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00279 01 LCI

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies on Mucus Glycoproteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael Kaliner	Senior Investigator	LCI, NIAID
OTHERS:	James Shelhamer	Senior Investigator	ICU, CC
	Zvi Marom	Staff Fellow	LCI, NIAID
	Carl Patow	Staff Fellow	SB, NCI

COOPERATING UNITS (if any)

Linda Steel Ph.D. AFRBI, Bethesda, Md.

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Laboratory of Clinical Investigation

SECTION
Allergic Diseases

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Md 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	2	0

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The secretion of mucus is a major determinant of both morbidity and mortality in asthma and other respiratory diseases. In order to examine mucus secretion, culture of respiratory tissues secreting biosynthetically-labeled mucus glycoprotiens has been examined. Anaphylaxis of airway or nasal mucosa leads to mucus release and the production of 5-HETE, 12-HETE and 15-HETE. Each of those HETE's is a mucus secretagogue as are SRS-A, histamine, cholinergic and α-adrenergic agonists, several prostaglandins and cyclic GMP. Prostaglandin-generating factor of anaphylaxis (PGF-A) also generates both mucus and HETE formation and may contribute to the mucus production seen in anaphylaxis. Phagocytosis of particles by macrophages leads to the elaboration of a low molecular weight, non-lipid material which is a potent stimulator of mucus secretion, which has been termed macrophage mucus-secretagogue (MMS). MMS release is seen from pulmonary alveolar macrophages as well as peripheral monocytes in response to zymosan ingestion or Staph protein A interactions. Mucus secretion from nasal and airway mucosa is inhibited by both corticosteroids and tetracycline. The combination of these agents acts in an additive fashion and has direct therapeutic implications.

LABORATORY OF IMMUNOGENETICS
1982 Annual Report
Table of Contents

Z01-AI Project Number		Page
Summary Report		
00166-05	Characterization of Rabbit and Human MHC Antigens	19-7
00167-05	Mass Spectrometry and Sequence Analysis of Polypeptides	19-8
00168-05	Immunoglobulin Allotypes of Heavy and Light Chains	19-9
00169-05	Primary Structural Analysis of Murine and Human Transplantation Antigens	19-10
00170-05	Serologic Studies of Human Histocompatibility Antigens	19-11
00171-05	Genetic Studies on Rabbit Immunoglobulins and Other Serum Proteins	19-12
00172-05	Structure and Synthesis of Peptides	19-13
00173-05	Control of Gene Expression	19-14
00180-04	Interspecies and Human-Human Hybridomas and Monoclonal Antibodies	19-15
00191-04	Immunoregulation of T Lymphocytes - The Role of Anti-Idiotype and receptor-anti-receptor complexes	19-16

Annual Report of the Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, NIH, PHS, HHS.

October 1, 1981 to September 30, 1982

Research Progress

The major subject of study within the laboratory of Immunogenetics pertains to molecules relevant to immune function and the genes that encode these molecules. Techniques in immunology, protein chemistry, cell biology, and molecular biology are currently being used to approach a variety of related problems. Systems currently under study include the MHC antigens and other lymphoid cell surface antigens of the human, mouse and rabbit; immunoglobulin molecules and genes of the human and the rabbit are being studied in order to obtain structural and regulatory information concerning antibody synthesis. These studies aim at a molecular description of various aspects of the immune system.

Human Histocompatibility Antigens. In preliminary studies on human class-I and class-II antigens a radioimmune assay based on binding of monoclonal antibodies to fixed cell preparations was devised (Quijano). The assay was used to monitor isolation of HLA-A,B,C or HLA-DR antigens using lectin columns as well as columns consisting of monoclonal antibodies coupled to solid supports. These assays indicated optimum procedures for isolation of antigenically active molecules.

In conjunction with the studies of isolation of antigenically active molecules a microcytotoxicity test was devised (Sachs, Kuo) to determine the alloantigenic determinants present on the molecules separated by the various techniques. Such testing is necessary in order to correlate the alloantigens present on the various molecules encoded within HLA-DR region with those recognized by monoclonal antibodies. Because the alloantisera are generally to weak to be used for isolation it is necessary to test the antigens following isolation using lectins or monoclonal antibodies. These assays will be used to isolate milligram quantities of human cell-surface antigens (Primerano, Li).

Genetic studies of human HLA-DR antigens will be carried out using cell lines derived from families with well charactertized crossovers between genes in the HLA region (Robinson). Family members have been typed using antisera directed against all known antigens within the D region and cells from the individual carrying the recombinant genes were used to prepare virally transformed cell lines that can be used for detailed studies of the antigen. In addition, family members carrying the normal (non-crossover) genes will be analyzed in a similar way to determine the differences related to the genetic crossovers. These studies will involve characterization of cell surface proteins from the family members and will include studies using recombinant DNA techniques such as Southern blot analysis of the DNA from the various family members.

In studies aimed at preparing monoclonal antibodies directed against HLA linked cell surface antigens, a novel B cell antigen was observed which serves as a marker for certain neoplastic and differentiated B cells (Marti). The antibody, designated 33.1, does not bind to normal human peripheral blood cells but reacts with practically all (55/60) B cells that have been tranformed with EBV. Atlhough the molecules recognized by monoclonal antibody 33.1 are similar in two-chain structure to human HLA-DR antigens, there are several significant differences. First of all, 33.1 is not present on normal macrophages or on B cells in normal lymphoid populations as is the DR antigen. In addition, T cells activated by various mitogens are negative for 33.1 whereas they are positive for DR-antigens. By contrast, activation of B cells with pokeweed mitogen or with dexamethosone convert normal B cells to 33.1 positivity. It is of interest that the 33.1 antigen is also carried by B cells from individuals with certain neoplastic disorders such as chronic lymphocytic leukemia and acute lymphoblastic leukemia. There is some evidence that the 33.1 antigen is polymorphic in that a number of cells from individuals with the DR type 7/7 have been found to be negative even after transformation with EBV.

Variant molecules of the human class-I histocompatibility antigen HLA-3 are being studied by protein structural techniques (Van Schravendijk, Coligan). Lymphoblastoid cell lines derived from indivduals showing differences in viral associated cytotoxicity that is restricted by the A3 molecule have been used as sources of the HLA-A3 antigen. Using a monocclonal antibody specifically directed against A3 it has been possible to isolate the A3 antigen from the variant sources and to obtain structural information. Peptide maps have been used and have revealed differences between the molecules from different sources. These studies are aimed toward a elucidation of functional sites within the class-I histocompatibility antigens.

Studies on Murine Class-I and Class II MHC Antigens. Continuing studies on murine class one antigens have revealed additional complexities in the structure of these molecules. It has been found that there are sequences within the K and D region-encoded molecules that may be specific for the various ends of the MHC. In addition, it has been found that the carbohydrate structure of the various MHC molecules may differ. Certain molecules have been found to have three glycosyl units, whereas others have only two. Comparison of the large amount of data available for murine class I antigens allow speculation concerning genetic mechanisms related to the synthesis and evolutionary origins of these molecules (Coligan, Maloy). In other studies, the class I antigens from various haplotypes which show strong crossreactivity one with the other are being compared by structural techniques (Lillehoj).

Studies on class II antigens of the mouse are being carried out in collaboration with investigators from NCI (Maloy, Coligan). These studies aim at determining how many molecules are encoded within the subregion of the H-2I region. Amino terminal sequence and two dimensional gel electrophesis techniques are being used to determine the complexity of the alpha and beta chains of the murine class two molecules.

Studies of Rabbit Histocompatibility Antigens. Recent studies of rabbit class one histocompatibility angitens have utilized a monoclonal antibody reactive with a 42,000 m wt molecule. This molecule reacts with a glycoprotein chain that is identical to the chain precipitated by anti beta-2-microglobulin. These identities were noted by amino terminal sequence analysis as well as by two dimensional gel elecphoresis (Wilkinson). However, in spite of this similarity the antigenic determinant recognized by anti beta-2-M and by the monoclonal antibody have different subcellular locations in a rabbit T cell line and in normal rabbit spleen cells. In addition, there is no ability of one antibody to clear for the other in normal clearance experiments. Therefore, it appears that there are two forms of the same class I molecule existing within the same cells. These studies are being continued using recombinant DNA techniques (Tykocinski). By use of recently developed mRNA cloning techniques, it has been possible to obtain a full length cDNA which encodes the rabbit MHC antigen. This clone is currently under sequence analysis and use of appropriately derived probes from it will allow elucidation of the structure of the rabbit MHC locus.

Cell Surface Markers for Rabbit Lymphoid Cells Subpopulations. A series of monoclonal antibodies were prepared against glycoprotein fractions derived from a rabbit T cell line (Wilkinson). A group of approximately 25 monoclonal antibodies was screened by immunoprecipitation and by cell sorter analysis for reactivity with various rabbit cell and tissue preparations. A group of four antibodies with unique reactivity were studied in great detail. Using these, it has been possible to define an antigen that is specific for the T cells of the rabbit as well as others that define specific subpopulations of B and T cells (Jackson). The molecular characterization of the antigens recognized by these monoclonal antibodies has begun and indicates that the T cell specific molecule is approximately 120,000 m wt and several of the other markers are 90,000. Further studies concerning rabbit T cells have included the induction and cloning of a rabbit T cell line specific for antigens related to streptococcal vaccines of the group C type. Specific reactivity to either the vaccine or the carbohydrate derived from the streptococcus is seen with this cell line in the presence of appropriate presentor cells.

Inter-species Hybridoma Cells. Studies of rabbit-mouse hybridomas secreting rabbit immunoglobulin chains have been underway in the laboratory for some years. Recently, it has been possible to obtain a cell line that is secreting an intact rabbit IgM molecule (Sogn, Kuo). This and other cell lines are being studied by karyotype analysis in order to determine whether the site of rabbit DNA integration into the murine genes is at a fixed point or if it is at random locations. In preliminary studies, no intact rabbit chromosomes have been found in the productive rabbit mouse hybridomas. The studies of interspecies hybrids are being extended to include the preparation of a mouse-human hybridomas using lymphoid cells derived from patients with various diseases in which auto-antibodies are produced (Marti, Sogn).

Other studies concerning hybridomas include attempts to prepare for human-human T cell hybridomas with various functional activities (Jackson). These studies are being carried out with investigators from the Cancer Institute and are utilizing cells obtained from patients with active EBV infection (Infectious mononucleosis).

Immunoglobulin Genes and Their Regulation. Studies of rabbit immunoglobulin genes have utilized rabbit-mouse hybridomas as a source of mRNA to prepare cDNA for study of the rabbit immunoglobulin genes

(Dreher). It has been possible in this way to obtain cDNA for a full length copy of a rabbit kappa light chain gene. From this cDNA probes have been constructed for V,J and C regions. These probes have been used to study the structure of the genes present in genomic libraries obtained by cloning DNA from rabbit sperm in bacteriophage (Emorine, Max). Preliminary evidence indicated significant differences between the structure of the immunoglobulin genes of the rabbit from those of the mouse and human which have been previously studied in some detail.

Other experiments concerning immunoglobulin genes relate to control of expression of genes encoding a human light chain (Max). These studies will utilize leukemia cells which represent B cell lines in various stages of differentiation. Because there is evidence of methylation of certain genes in activated cells, rectriction enzymes recognizing sites of methylation and those which do not recognize similar sequences when methylated will be used to compare DNA from cells of interest. These studies aimed to determine the structural differences between a cell that is actively synthesizing from a given gene and those containing the gene in an inactive synthesis state.

Functional studies of murine T cells. A number of T cell hybridomas in T cell lines with specificities for various antigens (ova, insulin, HSA) as detected by helper or suppressor function are under study (Folks). An assay system whereby the activity of the clones as helper or suppressor could be assayed has been devised and is based on an ELISA rather than on placque forming techniques. A number of antigen specific T cell suppressor lines are now available and have been screened for various functions following antigen presentation (Folks, Schroer).

Collaborative experiments with members of the lab of Immunology are investigating functional activity of anti-insulin T hybridomas. It is hoped to obtain antigen-specific secreted membrane molecules in these studies.

The Immune Response to Insulin. Hybridoma antibodies produced by 18 anti-insulin hybridomas (Schroer) have been studied. These antibodies are characterized with respect to their affinity and reactivity with insulins from various species and the reactive antigens on the insulin molecule have been identified. Anti-idiotype antisera have been prepared against a number of the antibodies and have been used to look for crossreactivity of various anti-insulin molecules. In collaborative studies with the diabetes branch of NIAMDD, the anti-insulin hybridomas are being used to determine whether any or all of them can bind to insulin when it is associated with the insulin receptor on fat cells. The use of these well characterized antibodies has the potential to determine functional aspects of the insulin molecule.

Administrative Report

Within the period covered by this report a number of personnel changes have taken place in the Laboratory of Immunogenetics. Dr. Gerri Marti, a commissioned officer, has joined the clinical center, Dr. Claudia Quijano, a visiting fellow for two years in LIG will continue in the Cancer Institute for a third year as a visiting fellow. Dr. John Sachs will leave October 1 to resume his professorship at University College in London. Dr. Richard Goldsby left the laboratory in January to resume teaching duties at the University of Maryland after six months as a visiting scientist. Dr. Henry Krutzsch who has carried out a program related to microsequence determination of proteins using gas chromatrography and mass spectrometry will leave in the fall to continue his research program at an institute within the area.

The laboratory has been joined by Dr. Maryann Robinson from Duke University and by Dr. Li XuanMao from the Second Medical College in Shanghai, Peoples Republic of China. Dr. Anna Maria Primerano has joined the Membrane Antigen Section and prior to October 1, it is expected that Dr. Maryiln Lightfoote and Dr. Elliott Cowan will join the laboratory. Dr. Lightfoote who is from the University of Virginia will join the Immunobiology section and Dr. Cowan from Washington University, St. Louis will join the Membrane Antigen Section.

There are plans to relocate the section of the LIG in Building 8 to Building 5. Rennovations are well underway and it is expected that the laboratory will move prior to the first of January. In addition, a synthesis section headed by Dr. W. Lee Maloy will begin in temporary quarters in Frederick, Maryland but move to Building 5 in the spring of next year.

Honors and Awards

During the past year reports of laboratory work have been given by Dr. Kindt whose served as the keynote speaker for the Immunobiology council of Johns Hopkins University and the University of Virginia at their annual meeting and at the meeting of the Chicago Immunology club. In addition, seminars of laboratory work were given by Dr. Kindt at Bowman-Gray Medical School, at Northwestern University, at the University of Illinois at Chicago, at the University of Alabama at Birmingham, at the University of Texas Southwestern Medical School, at the Bureau of Biologics in Bethesda, at the University of Paris and at the Pasteur Institute in Paris. Dr. Kindt has served as the program chairman for the American Association of Immunologists with responsibility for programming the 1982 meeting in New Orleans. He continues to serve as adjunct professor of Microbiology and Pediatrics at Georgetown University. Editorships currently held include the section editor for Immunochemistry of the Journal of Immunology, The Journal of Experimental Medicine, Proceedings for the Society of Experimental Biology and Medicine and Immunology and he is editing a volume of Contemporary Topics in Molecular Immunology. Dr. Kindt has been accepted under the work-study program to spend a sabbatical year at the Pasteur Institute in Paris.

Dr. Coligan served as chairman of a MiniSymposium at the annual meeting of the American Assocation of Immunologists and has presented a seminar of his work at the University of Florida. He is an invited speaker at a meeting on protein chemistry held in Honolulu in August. Dr. Coligan has been appointed to the editorial board of the newly published journal "Survey of Immunologic Research". Dr. W. Lee Maloy and Dr. Mark Tykocinski were invited speakers at FASEB mini symposia in April. Dr. Maloy and Dr. Susan Jackson were elected to membership this year in the American Association of Immunologists.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AI-00166-05 LIG

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Characterization of Rabbit and Human MHC Antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dr. Thomas J. Kindt	Chief, Lab	LIG, NIAID
Other:	Dr. Mark Tykocinski	Med. Staff Fellow	" "
	Dr. Maryann Robinson	Staff Fellow	" "
	Dr. Claudia Quijano	Visiting Fellow	" "

COOPERATING UNITS (if any)
Dr. Erich Long, Dr. B. Mach, University of Geneva
Dr. Armead Johnson, Georgetown University

LAB/BRANCH
Laboratory of Immunogenetics

SECTION
Immunogenetics Research Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.8	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Structural studies on rabbit MHC antigens have used monoclonal antibodies directed against a 42,000 m wt molecule that is not associated with beta-2 microglobulin (B_2m). Although no structural difference between the molecules associated with or not associated with B_2m were revealed by amino terminal sequence analysis or by two dimensional electropheresis, the B_2m determinants and those recognized by the antibody were in distinct subcellular locations as revealed by electronmicroscopy. In continuing studies of rabbit MHC genes using recombinant DNA techniques a full length cDNA copy of a rabbit class I antigens has been cloned and sequenced. Studies on human MHC antigens will utilize cells from families with well characterized crossovers in the MHC region. The HLA-DR antigens of appropriate family members will be analyzed by protein structural techniques and the HLA genes of these families further characterized in collaborative studies by recombinant DNA techniques.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AI-00167-05 LIG

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mass Spectrometry and Sequence Analysis of Polypeptides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dr. Henry Krutzsch	Expert	LIG, NIAID
Others:	Dr. Thomas J. Kindt	Chief	" "
	Dr. John A. Sogn	Res. Chemist	" "

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Immunogenetics

SECTION
Immunogenetics Research Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 2.3	PROFESSIONAL: 1.4	OTHER: 0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In immunogenetic and other studies, sensitive and rapid methodologies for peptide and protein structural analysis are needed. Techniques utilizing mass spectrometry are promising routes to this goal. A versatile, rapid and sensitive approach using dipeptidyl peptidases (DP) and gas chromatography-mass spectrometry (GC-MS) has been developed that can analyze picomolar quantities of peptides from the N- or C- terminus. Data handling has been enhanced and simplified by computer programs. Most recent research emphasis has been placed on the C-terminal sequencing aspect of the DP/GC-MS methodology. A number of peptides, with both free and blocked N-terminii, such as viral and tryptic peptides, have been successfully sequenced by this means. Purification schemes for the enzymes used have also been defined. Currently, several proteins are under study using the DP/GC-MS methodology.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AI-00168-05 LIG

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immunoglobulin Allotypes of Heavy and Light Chains

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dr. Thomas J. Kindt	Chief, LIG	LIG,	NIAID
Others:	Dr. Edward Max	C.O.	"	"
	Dr. Kevin Dreher	Staff Fellow	"	"
	Dr. Laurent Emorine	Visiting Fellow	"	"

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Immunogenetics

SECTION
Immunogenetics Research Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 2.3	PROFESSIONAL: 1.6	OTHER: 0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Rabbit mouse hybridomas (RMH) have been used in a number of studies concerning the structure and synthesis of rabbit immunoglobulin chains. This study has involved determination of conditions for stabilization of RMH and determination of growth conditions for maximal yields of mRNA. The rabbit L chain product of RMH 12F2 has been characterized by protein sequence analysis. This product could be precipitated from cell-free translates using mRNA from the 12F2 cell line. Following enrichment the mRNA was used to prepare cDNA which is full length copy including the variable region and leader sequence of this chain. An RMH producing a rabbit heavy chain is now under study. Additional studies are directed towards determining whether rabbit DNA integrates into the mouse myeloma cells randomly or at predetermined sites in the mouse genome.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AI-00169-05 LIG

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Primary Structural Analysis of Murine and Human Transplantation Antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dr. John E. Coligan	Res. Microbiologist	LIG,	NIAID
Others:	Dr. W. Lee Maloy	Sr. Staff Fellow	"	"
	Dr. M. Van Schravendijk	Visiting Fellow	"	"
	Dr. A.M. Diaz-Primerano	Visiting Fellow	"	"
	Dr. Erik Lilliehoj	Guest Worker	"	"

COOPERATING UNITS (if any)

D. Sachs, NCI, NIH, B. Biddison, NINCDS, NIH

LAB/BRANCH
Laboratory of Immunogenetics

SECTION
Membrane Antigen Structure

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	2.5	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Membrane molecules of murine and human origin are isolated and characterized with emphasis on molecules involved in the immune response. The goal of these studies is to gain an understanding of their function in molecular terms as well as to gain knowledge of their evolutionary relationships. Project areas include: (1) studies on murine H-2 and I a major histocompatibility complex antigens, (2) structural studies on human HLA-variant molecules and (3) Preliminary structural studies on other murine (Ly6.2) and human lymphocyte antigens (OKT3 series). Studies on H-2 antigens have resulted in the extensive sequence information for H-2K^b, K^d, D^b and D^d. These data revealed areas of polymorphism, sites glycosylation, location of disulfide bridges and variation in chain length as well as other interesting characteristics of these molecules. Studies on Ia are seeking to prove whether or not two structurally distinct IE molecules are present in the mouse.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AI-00170-05 LIG

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Serologic Studies of Human Histocompatibility Antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dr. John A. Sachs	Expert	LIG,	NIAID
Others:	Dr. Mei-Chang Kuo	Guest Worker	"	"
	Dr. Maryann Robinson	Staff Fellow	"	"
	Dr. Claudia Quijano	Visiting Fellow	"	"

COOPERATING UNITS (if any)

Dr. Armead Johnson, Dr. James Woody, Georgetown University

LAB/BRANCH
Laboratory of Immunogenetics

SECTION
Immunobiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 4.0	PROFESSIONAL: 2.0	OTHER: 2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Cell membrane HLA-A,B,C and DR like products have been prepared by conventional means from EBV tranformed cells homozygous for HLA-D region antigens. The glycoprotein fractions were further purified by immunoprecipitation and affinity chromatography with monoclonal antibodies monomorphic and polymorphic for Class II determinants. Further studies along the same lines with Class II allo-antisera have just now been initiated.

The eluted products have been monitored for specificity by two newly developed techniques: inhibition of microlymphocytotoxicity for HLA-DR like activity and inhibition of RIA for cross reactivity with other class II framework monoclonal antibodies. The direction of the project is to ally the structural determinants of Class II antigens revealed by immunochemical techniques with their serologic and cellular functions.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AI-00171-05 LIG

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Genetic Studies on Rabbit Immunoglobulins and Other Serum Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dr. John A. Sogn	Chemist	LIG, NIAID
Others:	Dr. Susan Jackson	Staff Fellow	" "
	Dr. Mark Tykocinski	Med. Staff Fellow	" "

COOPERATING UNITS (if any)

T. Chused, LMI, NIAID, J. Coe, Rocky Mountain Laboratory, NIAID, P.A. Cazenave, Institut Pasteur, Paris, France

LAB/BRANCH
Laboratory of Immunogenetics

SECTION
Immunogenetics Research Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 2.4	PROFESSIONAL: 1.6	OTHER: 0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A set of monoclonal antibodies prepared has been used in biochemical and functional analysis of rabbit lymphoid cell subpopulations. Detailed study has defined a small group of the monoclonal antibodies which recognize distinct cell surface molecules and define among them subsets of T cells in the thymus and periphery as well as subsets of B cells. Immunoprecipitation of labeled surface molecules has defined the antigens while the cell sorter has been used for cellular analysis. A number of antigen-specific rabbit T cell lines have been developed to characterize rabbit T cell responses to the group C streptococcus. These lines are being propagated for studies on antigen-binding molecules and restriction in antigen presentation. The kappa chain allotypes of wild rabbits (b97, b98) are under structural study in collaborative experiments. The hamster serum protein FP (female protein) has also been partially characterized in sequence studies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AI-00172-05 LIG

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Structure and Synthesis of Peptides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dr. John E. Coligan	Section Head	LIG, NIAID
Others:	Dr. W. Lee Maloy	Staff Fellow	" "
	Dr. Marie R. Van Schravendijk	Visiting Fellow	" "

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Immunogenetics

SECTION
Membrane Antigen Structure

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Through protein and DNA sequence analyses, much information on the primary structure of membrane bound proteins has accummulated over the past several years. It is proposed to utilize this information to prepare antibodies reactive with defined portions of these molecules. This will be done by synthesizing 10-20 residue peptides that correspond to sections of the sequence of the membrane bound proteins. After coupling to a carrier protein, antisera to these peptides will be prepared. In the case of transplantation and other lymphocyte antigens, these antibodies will be used to define sites of cellular recognition and serological specificities. The feasibility of utilizing synthetic peptides as protective immunogenic and therapeutic reagents will also be investigated. In these cases, peptides corresponding to portions of viral or parasitic proteins will be used to seek protective immunity against infection or to treat infection. By using small, defined peptides, it is potentially possible to limit the side effects encountered with vaccines.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AI-00173-05 LIG

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Control of Gene Expression

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dr. Edward Max	CO	LIG,	NIAID
Others:	Dr. Kevin Dreher	Staff Fellow	"	"
	Dr. Laurent Emorine	Visiting Fellow	"	"
	Dr. Mark Tykocinski	Med. Staff Fellow	"	"

COOPERATING UNITS (if any)

Dr. Stanley Korsmeyer, NCI, NIH

LAB/BRANCH
Laboratory of Immunogenetics

SECTION
Immunogenetics Research Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.8	2.0	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have obtained from a rabbit-mouse hybridoma a cDNA clone whose sequence agrees with the available amino acid sequence of the kappa light chain secreted by the hybridoma. Using variable and constant region fragments of the cDNA clone as probes several clones hybridizing to each probe have been obtained from a rabbit sperm genomic library These clones are currently being characterized.

The activation of specific genes in B cell development are being studied using DNA methylation analysis and DNAase sensitivity. In collaboration with Dr. Stanley Korsmeyer of NCI, several leukemia/lymphoma lines representing B-lineage cells at different stages of differentation are under analysis using probes for kappa, epsilon and mu immunoglobulin genes. In order to study a non-immunoglobulin gene whose activity may be developmentally regulated we are attempting to clone the human gene for the J chain which links IgM monomers. A human clone which cross-hybridizes with the mouse J chain gene is currently being sequenced.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00180-04 LIG

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Interspecies and Human-Human Hybridomas and Monoclonal Antibodies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dr. John A. Sogn	Chemist	LIG, NIAID
Others:	Dr. Gerald Marti	C.O.	" "
	Dr. Thomas Folks	Staff Fellow	" "
	Dr. Susan Jackson	Staff Fellow	" "

COOPERATING UNITS (if any)

G. Tosato, NCI, NIH, R. Yarchoan, NCI, NIH

LAB/BRANCH
Laboratory of Immunogenetics

SECTION
Immunogenetics Research Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 2.6	PROFESSIONAL: 1.6	OTHER: 1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Monoclonal antibody 33.1, prepared against a human EBV-transformed B cell line has been characterized in detail. The 33.1 antigen is undetectable on normal peripheral blood mononuclear cells but is present on most EBV-transformed B cell lines and B-CLL cells, on some other B cell-derived neoplastic cells and on normal B cells activated with EBV, pokeweed mitogen and dexamethasone. The antigen is a two-chain molecular with clear homology to human HLA-DR antigens but it is distinct from DR in structural features and in cellular distribution.

Rabbit-mouse Hybridoma H105-14 has been shown to secrete a 19S IgM with H and L chains of rabbit origin. The source of the presumed J chain (rabbit or mouse) is under investigation. Hybridoma H89, which expresses no group a allotype but appears to secrete an a2/H chain by sequence is being analyzed. Human-human T cell hybridomas prepared in collaborative experiments from cells of infectious mononucleosis patients have been shown to have nonspecific antibody-suppressing activity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AI-00191-04 LIG

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Immunoregulation of T lymphocytes - The Role of Anti-Idiotype and receptor - anti-receptor complexes.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dr. Kenneth W. Sell	Head	LIG, NIAID
Others:	Dr. Thomas M. Folks	Sr. Staff Fellow	" "
	Dr. Joyce A. Schroer	Expert	" "

COOPERATING UNITS (if any)
Dr. Ethan Shevach, LI, NIAID

LAB/BRANCH
Laboratory of Immunogenetics

SECTION
Immunobiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.8	1.2

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

T cell lines and T cell hybridomas for ovalbumin and beef insulin have been produced and tested for specificity by proliferation, radiobinding and helper/suppressor assays. Characterization of the factor(s) released by these cell lines is currently underway. For the insulin system, B cell hybridoma antibodies are being used to prepared idiotypic reagents which will be tested for T cell reactivity. In other studies being initiated, patients with recurrent herpes will be characterized in terms of their cellular immune status to HSV-2.

PHS-6040
(Rev. 2-81)

LABORATORY OF IMMUNOLOGY
1982 Annual Report
Table of Contents

Z01-AI Project Number		Page
Summary		20- 1
00030-14	Antigen Recognition and Activation of Immunocompetent Cells.----Paul	20-10
00035-07	Specificity in Immune Responses.----Inman	20-11
00036-17	Immunoglobulin Genetics: Ontogeny and Differentiation of Cells of the Rabbit Immune System.----Mage	20-12
00037-15	Immunogenetics of Mouse Immunoglobulins and Genetic Control of Antibody Response.----Lieberman	20-13
00038-10	Structure and Activity Studies on Immunologically Important Cells and Proteins.----Waxdal	20-14
00040-08	Genetic Control of Immunocompetent Cell Interactions.----Shevach	20-15
00147-07	The Mechanism of Activation of Thymus-Derived Lymphocytes.----Schwartz	20-16
00148-07	Lymphocyte Interactions, Receptors and Functions. ----Green	20-17
00223-01	Cellular Interactions in the Immune Response. ----Schwartz	20-18
00224-01	Monoclonal Antibodies as Probes for Understanding Cell Activation.----Shevach	20-19
00225-01	The Effects of Lymphokines on Non-Lymphoid Tissues.----Green	20-20
00226-01	Rabbit Ig Allotypes: Structure, Organization, and Regulated Expression of Ig Genes.----Mage	20-21
00227-01	Biochemistry of Lymphocyte Activation.----Waxdal	20-22
00228-01	Membrane Associated Glycoconjugates and Receptors as Differentiation Markers and Mediators of Cell-Cell Recognition and Communication.----Waxdal	20-23
00229-01	Studies on Lymphocyte Differentiation.----Davis	20-24
00259-01	Ia Molecules and Immune Response Genes.----Paul	20-25

PHS-NIH
Summary Statement
Office of the Chief
Laboratory of Immunology
October 1, 1981 through September 30, 1982

Introduction

The Laboratory of Immunology is concerned with the elucidation of the fundamental mechanisms underlying immunologic responses. It has made rapid progress through the use of three new technologies which are creating a revolution in immunologic sciences. These are the use of monoclonal antibodies, the adaptation of techniques of molecular genetics to immunologic problems, and the use of long-term lines of cloned normal and transformed lymphocytes. The continued use and improvement of these approaches should allow solution of many of the major problems which have concerned immunologists and should provide important approaches to the more precise regulation of the normal and the disordered immune response.

Identification of Genes Uniquely Expressed in Lymphocytes of Specific Differentiated States

The phenotype of any individual cell depends upon those genes which it has expressed. To a large extent, the differences in function and structure of two closely related cells are determined by the genes expressed in the mRNA of one cell but not another. Laboratory of Immunology scientists have examined the expressed genetic differences in lymphoid cells of distinct differentiated states by preparing cDNA probes which hybridize specifically with mRNA of one but not another member of a pair of cells. For example, cDNA prepared from the mRNA of a B cell tumor was extensively hybridized with mRNA from a T cell tumor. The single stranded material represents a "B cell specific" probe (B^x-T); similarly, T^x-B and $T_h{}^x$-T_s probes have been prepared. Based on the hybridization kinetics of these probes, it has been estimated that B-T and T-B differences are ∿1.7-2.4% of the total mRNA and correspond to about 200 distict genes expressed in one cell type but not the other. An analysis of the difference in expressed genes between a helper T cell hybridoma line and a suppressor T cell hybridoma line indicates that these differ from one another in about 0.3% of their expressed genes, corresponding to approximately 40 different genes. The number of these genes is sufficiently small that their isolation and characterization should be feasible. Selected cDNA's (B^x-T and T^x-B) have been cloned to prepare cell type-specific cDNA libraries. The availability of such libraries, coupled with specific probes, should greatly simplify the task of isolating each gene. Among the things such libraries have been used for thus far is to identify X chromosomal genes of the B^x-T or T^x-B type. This appeared particularly interesting because of the existence of multiple distinct human and mouse immunodeficiencies controlled by X chromosomal genes. One X chromosomal gene, pTX-1, identified in a T^x-B cDNA library, exists as a gene family. This cDNA hybridizes with 10 or 12 restriction fragments from the mouse X chromosome; pTX-1 appears to be expressed in T helper cells and not T suppressor cells. Due to the importance of gene families among other genetic systems involved in immune responses (i.e., immunoglobulins, Class I MHC molecules, γ-interferons), this X chromosomal set of genes merits considerable attention; it may exert a critical role in lymphocyte differentiation (Davis, Cohen, Hedrick, Heber-Katz, Schwartz and Paul, LI/NIAID; Kim and Asofsky, LMI/NIAID; Hansburg, LCB/NCI; D'Eustachio, Yale University).

Cloning and Characterization of cDNA containing DNA Sequences for Rabbit κ Light Chains and μ Heavy Chains

The understanding of the organization and regulation of expression of rabbit immunoglobulin (Ig) genes may lead to important general insights because of several very interesting features of rabbit Igs, including the expression of *a* locus allotypes on Igh-V regions, the phenomenon of hidden or latent allotypes and the capacity to induce long-term allotype suppression. In order to realize the promise of this system, it will be necessary to examine the genetic organization of the rabbit Ig regions by recombinant DNA technology. To accomplish this, a partial cDNA library was constructed from total spleen poly A(+)-RNA of an a3b5 del2,15 rabbit that had been hyperimmunized with S. *phneumoniae*, type III. Recombinant clones containing cDNA sequences of rabbit γ heavy (H) chain and κ light (L) chain were identified by a combination of colony hybridization, mRNA hybridization selection and translation, and Northern analysis; the correctness of identification was verified by sequencing. Rabbit cDNA clones were also obtained by cross hybridization with a mouse genomic probe. Several interesting features have already emerged from a study of the sequences of these cDNAs. There is an unexpectedly high degree of sequence homology between the 3' untranslated (UT) region of the cDNA's of b4 and b5 allotype; this homology appears to be greater than that of the coding sequences of the b4 and b5 *Igκ-C* genes. The rabbit cDNA μ clone is essentially a full-length copy of μ mRNA, including 5' UT, 5' leader, V_H, D, J, C_m, secretory tailpiece, and 3' UT regions. This cDNA has a series of codons for amino acids characteristic of the a2 allotype in the first and third FR. The cDNA sequence and the translated protein sequences show a high degree of homology for human and mouse m chains. These probes and additional ones, being prepared, should allow key insights into the organization of the rabbit Ig genes(Fitzmaurice, Pavirani, Bernstein and Mage LI/NIAID).

B Cell Growth Factor (BCGF) and Interleukin 1 (IL-1) are Critical for the Proliferation of Anti-m Stimulated B Cells

Antibodies specific for the membrane immunoglobulins (mIg), which act as B cell receptors for antigen, are powerful stimulants of B cell activation and prepare these cells to proliferate. If B cells are highly purified and cultured at low cell density (i.e., $1x10^4$/0.2 ml), the entry of anti-IgM stimulated B cells into the S phase of the cell cycle requires two factors. One of these factors is a T cell product. It has been identified in supernatants from activated T lymphoma and hybridoma lines, such as EL-4, and from long-term lines of non-transformed T cells of the Kimoto-Fathman type. This factor, which has been designated B cell growth factor (BCGF), is a molecule with an Mr of ∿18,000. It is distinct from interleukin-2 (IL-2) and other previously recognized lymphokines. BCGF may be separated from IL-2 by gel filtration, isoelectric focusing, phenyl-sepharose chromatography, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. BCGF is not bound by cells which express receptors for IL-2. The second factor is a product of macrophages and monocytes; it appears to be identical to interleukin 1 (IL-1). The identification of the macrophage factor critical to B cell proliferation as IL-1 is based on the finding that preparations of mouse and human IL-1, purified by distinct strategies, are rich in B cell co-stimulator activity and that when fractions from the last purification step of both purifications are analyzed, IL-1 and B cell co-stimulator activity comigrate. Although B cells stimulated to proliferate by anti-IgM, BCGF and IL-1 do not secrete Ig, they are prepared to do so if provided with two additional factors, tentatively designated EL-TRF and B15-TRF (Howard, Nakanishi and Paul LI/NIAID; Farrar LMI/NIDR; Takatsu and Hamaoka, Osaka University, Osaka, Japan).

Development of a Monoclonal Antibody to a T Cell Surface Component Critical for Interleukin-2 Dependent Cell Growth

Very substantial progress has been made in understanding the basis of T cell growth, including the identification of a specific growth factor, interleukin 2 (IL-2), and the identification of receptors for this growth factor which appear on the surface of activated T cells. However, it is clear that membrane components other than the IL-2 receptor itself play critical roles in IL-2 dependent T cell activation. Such a component has now been identified through the characterization of a monoclonal antibody which recognizes an antigenic determinant preferentially expressed on activated guinea pig T cells. This reagent (5C3) totally inhibits IL-2 mediated activation of T cell blasts and has powerful suppressive effects on the responses of primed T cells to alloantigens and to protein antigens. It only partially inhibits mitogen-stimulated T cell proliferation. 5C3 does not appear to be directed at the IL-2 receptor since it has no effect on the binding of radiolabeled IL-2 to its receptor and 5C3 negative blasts, produced by culturing T cells with concanavalin A in the presence of 5C3, absorb IL-2 activity although they fail to proliferate in response to IL-2. The precise role that the surface molecule recognized by 5C3 plays in IL-2 stimulated T cell proliferation is uncertain but this reagent should make possible a more complete understanding of this critical process (Malek and Shevach, LI/NIAID; Robb, Glenolden Laboratories, DuPont Co.).

Evidence for the Interaction of Antigen and Ia Molecules in Antigen-presentation

T lymphocytes recognize both antigen and Ia molecules. Specific immune response (Ir) genes function by determining what antigen-Ia pairs may be recognized. If the determination of which antigen-Ia pairs are "permissive" is dependent upon interaction between antigen and Ia molecules, one would anticipate that antigens which stimulate T cell responses should display two separate sites. One of these is the site through which the antigen is recognized by the T cell receptor, the other the site which interacts with the Ia molecule. A search for evidence for two sites has been made using a cyanogen bromide cleavage fragment of pigeon cytochrome c, encompassing residues 81-104. By determining the capacity of B10.A mice immunized with pigeon cytochrome c fragment to be stimulated by evolutionary variants of this fragment, it was clear that a glutamine residue at position 100 was critical and that serine could be interchanged for glutamine. The existence of a second site is strongly suggested by the finding that amidination of the fragment creates a peptide which does not cross-react with the native peptide but nonetheless leads to the priming of T cells which display a pattern of cross reaction with amidinated cytochrome c fragments from other animals which is essentially the same as the cross-reactivity pattern which exists among T cells specific for the non-amidinated fragment. Furthermore, the strain distribution of responsiveness to native and amidinated pigeon cytochrome c peptides is the same despite the lack of cross-reactivity of these peptides and the same monoclonal anti-Ia antibody blocks responses to both antigens. These results suggest that the site defined by the common features of the native and amidinated fragments determine their capacity to be recognized by the Ia molecules of B10.A (i.e., define the "Ir site"). This site includes glutamine at position 100. The amidinated lysine residue, identified as lysine 99, appears to mark the site recognized by the T cell receptor. These results provide strong, indirect support for a role for Ia determined specific antigen presentation in Ir gene determined immune responses

(Schwartz and Inman, LI/NIAID; Hansberg and Appella LCB/NCI;Pincus LTB/NCI).

An independent line of evidence which also supports the concept that some type of *specific* interaction between antigen and Ia molecules is critical to the process of antigen-recognition by T cells comes from an analysis of the fine specificity of cloned T cell lines and T cell hybridomas specific for the carboxy terminal cyanogen bromide cleavage fragments of pigeon and moth cytochrome *c*'s. T cell clones from B10.A mice immunized with pigeon cytochrome *c* fragment can be activated by that antigen presented by syngeneic (B10.A) antigen presenting cells (APC) but fail to respond to pigeon cytochrome *c* presented by APC of the B10.A(5R) type. However, such clones will react to a cross reactive cytochrome *c* peptide (i.e., moth cytochrome *c* peptide) presented by B10.A(5R) APC. T cell clones of B10.A(5R) mice specific for moth cytochrome *c* peptide display essentially the same pattern of responsiveness; that is, the fine specificity of a T cell clone varies depending on the Ia alleles expressed by the APC. An entirely comparable set of results is observed with cytochrome *c* specific T cell clones from B10.S(9R) and B10.A mice. The change in fine specificity of antigenic recognition coincident with change in the specificity of the Ia molecules of the APC strongly suggests that it is a complex of the two elements which the T cell recognizes and that a change in the structure of one can be compensated for by an appropriate change in the structure of the other. (Heber-Katz, Hedrick, and Schwartz, LI/NIAID; Hansburg, LCB/NCI; Matis, Hecht and Longo, MB/NCI).

Production of Ia Mutants in B Cell Lines with Antigen Presenting Functions

Ia molecules are Class II major histocompatibility complex (MHC) gene products which function as restriction elements in the recognition of antigen by T lymphocytes and as immune response (Ir) gene products. Indeed, Ir gene product function appears to be related to the fact that there is specificity in the capacity of individual restriction elements to be co-recognized with certain antigens. That specificity may be completely imposed by the repertoire of T cell receptors expressed among mature T cell populations or may result from specific interactions between Ia molecules and antigens. Ia mutants could be very valuable tools in examining this issue since the changes in antigen-presenting properties which occur when individual amino acids at specific locations in the Ia molecule are changed should allow a determination of the nature of Ia-specificity. To study this, B lymphoma lines with antigen-presenting functions were identified. One such line, M12.4, was used to prepare B lymphoma -B cell hybridomas which expressed Ia antigens, restriction phenotype, and Ir gene functions of the normal cell partner. A B lymphoma-B cell hybridoma was prepared by fusing the H-$2^{d/d}$ B lymphoma subline M12.4.1 with B cells from a H-$2^{a/d}$ F_1 donor. This line, TA3, is potentially valuable for selection of mutants because it can be expected to express only one copy of the H-2^a MHC. TA3 cells were mutagenized with ethyl methane sulfonate and the surviving cells lysed with complement and a monoclonal antibody (10.2.16) specific for determinant on the I-A^a molecule. The few surviving cells were stained with a second monoclonal antibody (24.7.11) specific for a distinct determinant on the I-A^a molecule and positive cells selected by an electronic cell sorting. A cloned cell line was prepared (A8) which was 24.7.11 positive and 10.2.16 negative. This line had lost the capacity to present the antigen hen egg lysozyme (HEL) to a H-2^a T cell hybridoma specific for HEL associated with an I-A^a restriction element but retained the capacity to present the terpolymer of glutamic acid, alanine, and tyrosine (GAT) to another H-2^a T cell hybridoma specific for GAT associated with an I-A^a restriction element. Responses to both HEL and GAT are determined by I-A^a Ir genes. Structural studies of the mutant I-A^a molecule and genes of A8 are now being undertaken in order to determine the structural basis of this change in antigen presenting properties. This approach has also been used to create a large number (>30) other mutants which are now being analyzed functionally and serologically (Glimcher, Green, Heber-Katz, Schwartz, and Paul LI/NIAID; Hamano, Kim and Asofsky LMI/NIAID; Sharrow and Sachs, IB/NCI).

Induction of Transplantation Tolerance by Splenic Allografts

The immunological events associated with long-term acceptance of non-syngeneic organ and tissue grafts are still poorly understood. A powerful model for the examination of this problem is provided by the observation that transplantation tolerance can be induced in strain 2 guinea pigs by grafting of spleens from strain 13 donors. A substantial fraction of such recipients accept their grafts and survive for long periods of time without clinical evidence of graft versus host disease (GVHD). Such animals have markedly diminished mixed lymphocyte reactivity toward stimulator cells of strain 13 type. These long-term recipients possess cells capable of suppressing responses of normal strain 2 T cells to strain 13 stimulator cells. Furthermore, transfer of spleen cells from strain 2 guinea pigs which have retained strain 13 allografts to normal strain 2 guinea pigs induces a state of transplantation tolerance in these otherwise normal recipients. Thus, strain 2 guinea pigs which received an intravenous transfer of cells from tolerant donors had profoundly suppressed anti-13 mixed lymphocyte responses and 83% of them failed to reject intraabdominal strain 13 cardiac allografts. Studies of the cellular basis of this transfer of transplantation tolerance are now in progress (Shevach, LI/NIAID; Bitter-Suermann, Dept. of Pathology, Georgetown University Medical School).

Interleukin 1 Produced by Silica Stimulated Human Monocytes Causes Fibroblast Proliferation.

Interleukin 1 (IL-1) is a potent, biologically active molecule, produced by activation of monocytes through immunological and non-immunological stimuli. Although IL-1 was initially recognized for its role in the activation of thymocytes and T lymphocytes, it is now clear it has important effects on non-lymphoid tissues. One of its most striking activities of this type is the induction of fibroblast proliferation. The demonstration that the factor responsible for fibroblast proliferation is interleukin 1 is based on the careful examination of the capacity of material separated by a variety of procedures to stimulate thymocyte proliferation in the presence of concanavalin A (an assay for IL-1 activity) and to cause fibroblast proliferation. The materials with these activities comigrate on SP-Sephadex cation exchange chromatography, on HPLC size exclusion chromatography, and on phenylsepharose chromatography. They have the same Mr (13,000) and each is found in three separable species on isoelectric focusing, with pIs of 5.2, 5.8, and 6.8. The induction of fibroblast proliferation by IL-1, and the increased collagen biosynthesis which also occurs, provides a clear explanation for the fibrosis known to occur at the site of many *in vivo* immune responses. Furthermore, it has now been shown that treatment of human monocytes with silica causes the secretion of IL-1 and this material expresses the capacity to stimulate fibroblast proliferation. This suggests that the fibroblast proliferation and fibrosis which occurs in chronic inflammatory diseases, such as silicosis, may result from the local production of interleukin 1. (Schmidt and Green, LI/NIAID; Oliver, LB/NHLBI; Gery, LVR/NEI)

P. falciparum Infected Erythrocytes Bind Specifically to Human Endothelial Cells and to an Amelanotic Melanoma Cell Line

An important cause of the pathologic features of P. falciparum malaria is the binding of infected erythrocytes to vascular endothelial cells. An *in vitro* model for this binding event has been developed through the collaborative efforts of scientists in the Laboratory of Immunology and the Laboratory of Parasitic Diseases. Aotus erythrocytes infected with mature asexual forms of P. falciparum attach to cultured human umbilical vein endothelial cells. This binding step is specific for both the infected erythrocyte and the endothelial cell. Electron microscopic studies indicate that the binding takes place through knobs found on infected but not normal erythrocytes. The more detailed study of this process, particularly the type of large scale preparations needed to isolate the membrane receptors and to prepare monoclonal antibodies against them are limited by the difficulty of growing large numbers of human umbilical vein endothelial cells. To circumvent this difficulty, many continuous cell lines were screened for the capacity to bind infected erythrocytes. An amelanotic melanoma cell line behaved identically to endothelial cells in its ability to bind malaria infected erythrocytes. The binding properties of the melanoma cells were the same as those of the endothelial cells and electron microscopic studies demonstrated that the knobs on the surface of the infected erythrocytes were the points of attachment to the melanoma cells. The availability of this cell line should now make possible rapid progress in receptor isolation (Schmidt and Green, LI/NIAID; Udeinya, Aikawa, Miller, Leech and Barnwell, LPD/NIAID; and Hay, American Type Culture Collection).

Honors, Awards and Scientific Recognition

Members of the Laboratory of Immunology play important roles in the national and international scientific community. Included in this is service on editorial boards of important journals. Dr. Paul is chairman of the editorial board of the *Journal of Immunology* and editor of the *Annual Review of Immunology*. He is an advisory editor of *the Journal of Experimental Medicine*, a member of the editorial boards of *Immunological Reviews*, *Human Immunology* and the *Journal of Clinical Immunology* and an associate editor of *Immunological Communications*. Dr. Green is a member of the editorial boards of *Clinical Immunology* and *Immunopathology* and of *Immunological Communications*. Dr. Shevach is a member of the editorial boards of the *Journal of Immunological Methods*, *Cellular Immunology*, the *Proceedings of the Society of Experimental Biology and Medicine* and the *Journal of the Reticuloendothelial Society*. Dr. Schwartz is an associate editor of the *Journal of Immunology* and a member of the editorial board of *Stem Cells*. Dr. Mage is a member of the editorial board of the *Journal of Immunologic Methods*. Dr. Inman is an advisory editor of *Molecular Immunology*. Dr. Germain is an associate editor of the *Journal of Immunology* and of the *Journal of The Reticuloendothelial Society*.

Dr. Paul was elected to membership in the National Academy of Sciences, U.S.A. and to honorary membership in the Scandinavian Society of Immunology. He is a councillor of the American Association of Immunologists and a member of the Board of Directors of the Foundation for Advanced Education in the Sciences. He is U.S. Chairman of the Immunology Board of the U.S.-Japan Collaborative Medical Science Program, and is a member of the Scholars Selection Committee of the Fogarty International Center, of the Committee on Promotions and Tenure, NIAID, of the Scientific Review Board of the Howard Hughes Medical Institute, of the Scientific Board of Visitors of the Oklahoma Medical Research Foundation, and of the Committee on Personnel for Research of the American Cancer Society.

Dr. Paul organized the first Immunology Symposium of the Immunology Board of the U.S.-Japan Collaborative Medical Science Program. He was an invited speaker at the Nobel Symposium on Genetic Control of the Immune Response in Stockholm, at the annual meeting of the Scandinavian Society for Immunology in Umeå, Sweden, at the Symposium on Control of Cell Growth at the FASEB meeting in New Orleans, at the Oji Symposium in Sapporo, Japan, and at the New York Academy of Sciences Conference on IgD.

Dr. Green was made a member of the Austrian Society for Allergy and Immunology. He is a member of the Board of Trustees of the American Academy of Civil Service Physicians. He completed a term on the program committee of the American Association of Immunologists, having served as its chairman and co-chairman, and is currently a member of the meetings committee of FASEB. In March, 1982, Dr. Green was Visiting Professor of Research at Harbor General Hospital in Torrance, California.

Dr. Mage was elected president of the D.C. Chapter of Sigma Xi in June, 1981, for a two year term. She was chairman-elect and then chairman of the American Society for Microbiology, Immunology Division. Dr. Mage was an invited lecturer for the Department of Biochemistry, East Tennessee University School of Medicine and the Department of Microbiology and Immunology at Wayne State University Medical School. She was organizer for the FAES Immunology 520M course in 1981-82 and 1982.

Dr. Shevach is a member of the Arthritis Foundation Fellowship Review Committee and the program committee of the American Association of Immunologists. Dr. Shevach was a lecturer for the Foundation of Microbiology for the academic year, 1982-83, a guest speaker at the annual meetings of the Reticuloendothelial Society and the American Society for Microbiology. He spoke, also, at the FASEB Conference on Immunopharmacology, the 5th Ir Gene Workshop, and in the IUIS course in immunology and infectious diseases cosponsored by the Mexican Society for Immunology in Mexico City.

Dr. Schwartz was an invited speaker at the Gordon Conference on Animal Cells and Viruses in Tilton, New Hampshire, June, 1982, a lecturer for the American Association of Immunologists' course on Regulatory Elements of the Immune System, and a minisymposium speaker at the FASEB meeting in New Orleans in March, 1982.

Dr. Inman was appointed to the Organizing Committee for the 5th International Symposium on Affinity Chromatography and Biological Recognition.

Dr. Waxdal was an invited symposium speaker at the Mid-West Autumn Immunology Conference in October, 1981, and at the 2nd International Conference on Immunopharmacology in July, 1982.

In addition, laboratory members presented seminars and lectures at universities and research institutes, both in the United States and abroad.

Administrative, Organization and Other Changes

The Laboratory of Immunology was successful in recruiting Dr. Ronald Germain from his position as Associate Professor of Pathology at Harvard Medical School. Dr. Germain was appointed as a senior investigator and is developing a research group aimed at studying the molecular genetics of Class II mouse major histocompatibility complex molecules, the biological functions of these molecules, mechanisms regulating antigen-stimulated T lymphocyte activation, and suppressor pathways in the immune system.

The Laboratory continues to play an important role in the training of young scientists. During the past year, Dr. Laurie Glimcher completed an appointment as a research associate, Drs. Patricia K.A. Mongini and Steven Zweig completed their appointments as guest workers, Dr. Claudine Kieda completed her appointment as a visiting fellow, and Dr. Benjamin Sredni returned to Israel after comleting his appointment as a visiting associate. Each of these young scientists made a very substantial contribution during their training period in the Laboratory. Drs. Kurt Gunter and Michael Norcross entered the Laboratory as medical staff fellows, Drs. Gen Suzuki and Zdenko Kovac were appointed to visiting fellowship positions and Dr. Drew Bentley became a Guest Scientist.

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U.S. DEPARTMENT OF
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PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00030-14 LI

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Antigen Recognition and Activation of Immunocompetent Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	William E. Paul	Chief	LI/NIAID
OTHERS:	Anthony DeFranco	Guest Worker	LI/NIAID
	John T. Kung	Visiting Associate	LI/NIAID
	Maureen Howard	Visiting Associate	LI/NIAID
	Kenji Nakanishi	Visiting Fellow	LI/NIAID

COOPERATING UNITS (if any) Elizabeth Raveche, LEP/NIADDK; Susan Sharrow, IB/NCI; John Farrar, LMI/NIDR; Patricia Mongini, Hospital for Joint Diseases, NY, NY; Eleanor Metcalf, Dept. of Microbiology, USUHS, Bethesda, MD.

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NIAID, NIH, Bethesda, Maryland 20205

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Resting B lymphocytes may be activated either by receptor-mediated stimulation, such as those provided by antigen or nti-IgM antibody, or by interaction with MHC restricted helper T cells. Both stimulants cause virtually all resting B cells to enter G_1. The entry of G_1 B cells of the Lyb5+ type into S phase is controlled by two soluble factors-one a T cell product, designated B cell growth factor (BCGF), and the second, the macrophage product, interleukin 1 (IL-1). BCGF is a newly described lymphokine distinct from other T cell products such as IL-2. The differentiation of B cells activated by anti-μ in the presence of BCGF and IL-1 into antibody secreting cells depends upon two distinct "T cell replacing factors (TRFs)," one found in the supernatant of the B151K12 hybridoma (B15 TRF) and the second in the supernatant of the T lymphoma EL4 (EL-TRF).

Studies of the class switching by Lyb5$^+$ B cells indicates they may use three distinct pathways in response to type 2 antigens - IgM→IgG_3; IgM→IgE; IgM→IgA.

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PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Specificity in Immune Responses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John K. Inman	Sr. Investigator	LI/NIAID
OTHERS:			

COOPERATING UNITS (if any)

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2	1	1

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The principal aim of this project is to test the hypothesis of general multispecificity for the combining regions of antibodies and other kinds of receptors. Receptor sites should be capable of interacting with an occasional, disparately structured substance with an affinity high enough to affect biological function. Radiolabeled antibodies or solubilized receptors are passed through small, affinity chromatography columns. Accurate measurements are made of the retention (retardation) caused by the affinity adsorbent in the presence and/or absence of many, diverse, suitably large compounds. The resulting retention values are employed directly in calculating association constants, whose occurence frequencies provide a description of a receptor's multispecific character. Monoclonal antibodies from myelomas and hybridomas are currently being studied. In this study, large affinity probes that can be covalently bound to column matrices are being synthesized by systematic routes involving techniques used in peptide synthesis. Multispecific interactions will be usefully employed in extending the scope of specific affinity based separations and assays. Multispecificity frequencies will play a role in understanding the specificity of immune responses.

PHS-6040
(Rev. 2-81)

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PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immunoglobulin Genetics: Ontogeny and Differentiation of Cells of the Rabbit Immune System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Rose G. Mage	Sr. Investigator	LI/NIAID
OTHERS:	N. McCartney-Francis	Guest Worker	LI/NIAID

COOPERATING UNITS (if any) W.E. Gathings and M.D. Cooper, Univ. Al., Birmingham, AL; R.R. Fox, The Jackson Lab., Bar Harbor, ME., S. Dray, K. Knight, A. Gilman-Sachs, and W.C. Hanly, U. Il., Chicago, IL., W.J. Mandy, Austin, TX, R. Hamers, C. Hamers-Casterman and J. Naessens, Brussels.

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1.5	0.5	1.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Our goals are to understand the genetics and organization of rabbit immunoglobulin genes and the mechanisms that regulate their expression during lymphoid cell differentiation. We define structural differences between genetically controlled rabbit Ig allotypes using antisera we prepare and characterize. We conduct breeding experiments with rabbits of defined allotypes and study their cells as they exist in vivo and after culture in vitro. In b4b5 rabbits suppressed for paternal allotype, cells with surface Ig of that type appear by 1 year and are found in proportions higher than are expressed in serum. A subpopulation of small pre-B cells (∿20%) express κ light chains and exhibit allelic exclusion. In heterozygotes, κ allotypes that are inbalanced in expression in adults are expressed in balanced proportions in pre-B and B cells of newborns. Mutant Basilea rabbits produce b9-like light chains detectable in their pre-B cells. Cell lines from rabbit splenocytes have been maintained in culture for more than 6 months, are being subcloned and characterized as B cells, and will be used for regulation, RNA and DNA studies. We have typed F2 and backcross progeny with antisera to bas kappa allotype and demonstrated that this trait behaves as an allele (or pseudoallele) of b4,b5 and b6 allotypes.

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U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00037-15 LI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immunogenetics of Mouse Immunoglobulins and Genetic Control of Antibody Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Rose Lieberman	Sr. Investigator	LI/NIAID
OTHERS:	S. Nishinarita	Visiting Fellow	LI/NIAID
	W. Humphrey, Jr.	Biology Technician	LI/NIAID
	W. E. Paul	Chief	LI/NIAID

COOPERATING UNITS (if any) M. Potter, LCB/NCI; L.A. D'Hoostelaire, Litton Bionetics, Rockville, MD; Salk Institute, La Jolla, CA.

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TOTAL MANYEARS: 3	PROFESSIONAL: 2	OTHER: 1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Wild mice from the Eastern Shore (ES) of Md. that fail to respond to phosphorylcholine (PC) immunization were mated to inbred mice and their progeny studied for 1) the immune response to PC; 2) the C3 and T15 allelic forms of anti PC antibodies described in inbred mice; and 3) the Igh-C allotypes. Wild mouse 378, phenotype PC T15 C3 Igh-C^a was bred to C.B20 (P$\overset{+}{C}$ T1$\overset{+}{5}$ C$\overset{+}{3}$ Igh-C^b) and then backcrosses (BC) were made to C.B20 and 378. Similar crosses of another (ES) mouse 645(PC T1$\bar{5}$ C$\bar{3}$ Igh-$C^{a,e,f}$) which appeared to be an Igh-C recombinant were made. 56/59 F_1 progeny of 378 and 645 bred to C.B20 failed to respond to PC, suggesting a dominant suppressor gene present in the wild mice. 49/51 progeny from BC to ES mice gave no PC response, suggesting control by at least 2 or more genes. 44/47 progeny from BC to C.B20 produced high anti-responses. The genetics of the expression of C3 and T15 allelic forms of anti PC antibodies appears to be more complex. F_1 progeny frequently expressed low titers of C31d in mice with no anti PC antibody. Occasionally, T151d also appeared. In F_1 mice BC to ES, a similar situation pertained, but less frequently. In x C.B20. C3 and T15 were present in the majority of the anti PC responder mice. There was no concordance of C3 and T15 expression and allotype.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00038-10 LI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Structure and Activity Studies on Immunologically Important Cells and Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Myron J. Waxdal	Sr. Investigator	LI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been divided into two new projects: Z01 AI 00227-01 LI and Z01 AI 00228-01 LI. The parent project has, therefore, been terminated.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00040-08 LI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Genetic Control of Immunocompetent Cell Interactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ethan M. Shevach	Sr. Investigator	LI/NIAID
OTHERS:	T. Malek	Guest Worker	LI/NIAID
	J. Jakway	Guest Worker	LI/NIAID
	G. Dos Reis	Visiting Fellow	LI/NIAID
	I. Y. Sillers	Visiting Fellow	LI/NIAID
	L. Glimcher	Research Associate	LI/NIAID
	H. Lillehoj	Staff Fellow	LI/NIAID

COOPERATING UNITS (if any) Dr. Hinrich Bitter-Suermann, Department of Pathology, Georgetown University Medical School, Washington, D.C.

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INSTITUTE AND LOCATION
NIAID/NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	3.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The long range goals of this project are to determine the role of I-region associated (Ia) antigens in the regulation of immunocompetent cell interactions and to determine the mechanism of action and site of expression of the major histocompatibility complex (MHC) linked immune response (Ir) genes. During the past year we have concentrated our studies on several different areas. We have: 1) demonstrated the existence of T cells of responder origin that recognize antigen in association with nonresponder macrophages. This result is most consistent with theories of Ir gene function based on the concept of a hole in the T cell repertoire taking place during the induction of self-tolerance; 2) constructed autoreactive murine T cell hybridomas which should allow us to precisely analyze the role of Ia antigens in the syngeneic mixed leukocyte reaction; 3) obtained evidence for macrophage processing of antigen which may occur at a site on the macrophage cell surface; 4) developed a model for the transfer of specific transplantation tolerance following induction of T suppressor cells by splenic allografting.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00147-07 LI

PERIOD COVERED
October 1, 1981-September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Mechanism of Activation of Thymus-Derived Lymphocytes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ronald H. Schwartz	Sr. Investigator	LI/NIAID
PTHERS:	Ellen Heber-Katz		LI/NIAID
	Steven Hedrick		LI/NIAID
	Jonathan Ashwell	Research Associate	LI/NIAID
	Lawrence Samelson	Research Associate	LI/NIAID
	John K. Inman	Sr. Investigator	LI/NIAID

COOPERATING UNITS (if any) Louis Matis, MB/NCI, Dan Longo, MB/NCI; Mimi Davis, MB/NCI; Matthew Pincus, LTB/NCI; Daniel Hansburg and Ettore Appella, LCB/NCI.

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NIAID/NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project focuses on the mechanism and function of immune response (Ir) genes in the activation of thymus-derived T-lymphocytes using a secondary T-cell proliferation assay and the technology of T-lymphocyte cloning. With these techniques we have demonstrated that Ia molecules and antigen interact during T cell activation. In particular, for the antigen, moth cytochrome c, two parts of the molecule have been distinguished, one which interacts with the macrophage Ia molecule, the other with the T cell receptor.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00148-07 LI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Lymphocyte Interactions, Receptors and Functions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ira Green	Sr. Investigator	LI/NIAID
OTHERS:	Laurie H. Glimcher	Research Associate	LI/NIAID
	Myron J. Waxdal	Sr. Investigator	LI/NIAID
	William E. Paul	Chief	LI/NIAID
	John Schmidt	Research Associate	LI/NIAID
	Louis Miller	Sr. Investigator	LPD/NIAID

COOPERATING UNITS (if any) Ada M. Kruisbeek, I/NCI

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NIAID/NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The goal of this project is to explore and understand immunological mechanisms that may be important in initiation or control of disease processes. We transformed by SV40 virus a cell type in the thymus which had many features of thymic epithelial cells, including morphology, both light and EM, and the presence of keratin. The cell surface had MHC antigens of Class I type. Supernatants of these cells were capable of inducing cytotoxic effector cell capacity in thymocytes. These cell lines may be important for delineating cell interactions that lead to T cell differentiation in the thymus. We are also investigating biochemical properties of tumor-associated transplantation antigen TSTA of guinea pig B cell L_2C leukemia of inbred Strain 2 guinea pigs, whose assay is immunization protection tests and is unusual in that it has a low M.W. and is resistant to boiling. It may represent a new class of antigens on normal B cells and once identification is completed, a similar class of materials could be looked for on human B cell malignancies. We are studying binding of P. falciparum infected erythrocytes to umbilical vein endothelial cells in culture, reproducing a process that occurs in vivo. We discovered that a human melanoma cell line also binds and will greatly expedite isolation of this receptor for malaria-infected red cells.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00223-01 LI

PERIOD COVERED

October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cellular Interactions in the Immune Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ronald H. Schwartz	Sr. Investigator	LI/NIAID
OTHERS:	Laurie H. Glimcher	Research Associate	LI/NIAID
	Ira Green	Sr. Investigator	LI/NIAID

COOPERATING UNITS (if any) Dan Longo, MB/NCI; Alfred E. Singer, IB/NCI

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INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.6	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project explores cellular interactions among T lymphocytes. During this year, the syngeneic mixed leukocyte response was examined by cell number-response titrations and limiting dilution analysis to determine the number and nature of the T cells involved in this apparent autoimmune response. Evidence was obtained for 3 interacting cell populations which recognize and respond by proliferation to Ia molecules on a syngeneic radioresistant 4th stimulating cell population. The particular Ia molecules which are recognized is determined in the thymus during T cell differentiation. One of the T cells is Lyt 1^+2^- and one or more of the T cells produces T cell growth factor. The frequency of one of the cells is 1/25,000, and the frequency of one other is approximately 10-fold higher. Thus, the syngeneic mixed leukocyte response appears to be a complex series of cellular interactions which could potentially provide a good model for a primary immune response *in vitro*.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00224-01 LI

PERIOD COVERED

October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Monoclonal Antibodies as Probes for Understanding Cell Activation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ethan M. Shevach	Sr. Investigator	LI/NIAID
OTHERS:	T. Malek	Guest Worker	LI/NIAID
	J. Jakway	Guest Worker	LI/NIAID
	G. Dos Reis	Visiting Fellow	LI/NIAID
	S. Zweig	Guest Worker	LI/NIAID

COOPERATING UNITS (if any) Dr. C. Raine, Department of Pathology, Albert Einstein College of Medicine, Bronx, New York

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NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1	1.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have developed a series of murine monoclonal antibodies to guinea pig T lymphocytes and accessary cells and used these antibodies as tools to further our understanding of the process of T cell activation by both non-specific and specific immunologic stimuli. Our studies have been concentrated in several different areas. We have 1) developed a monoclonal reagent to a cell surface antigen distinct from the receptor for the T cell growth hormone, interleukin-2 (IL-2), which plays a critical role in the activation signal transmitted by IL-2 to the T cell; 2) employed the immunosuppressive drug, cyclosporin A (CY A), as a probe for T cell activation and demonstrated that CY A inhibits both IL-2 production as well as IL-2 receptor induction. 3) analyzed the immunologic makeup of the central nervous system infiltrate in both acute and chronic experimental allergic encephalomyelitis (EAE); 4) identified monoclonal antibodies to human Ia antigens which cross react with guinea pig Ia and which recognize evolutionary conserved epitopes which play unique roles in T cell activation.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00225-01 LI

PERIOD COVERED
October 1, 1981-September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Effects of Lymphokines on Non Lymphoid Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ira Green	Sr. Investigator	LI/NIAID
OTHERS:	John A. Schmidt	Research Associate	LI/NIAID

COOPERATING UNITS (if any) Cynthia Oliver, IR/LB.NHLBI; Igal Gery, LVR.NEI

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NIAID/NIH, Bethesda, MD 20205

TOTAL MANYEARS: 1.8	PROFESSIONAL: 1.5	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Lymphokines are substances produced by lymphocytes and monocytes that have important functions in lymphoid cell interactions and regulation. Recently, we have observed that supernatants of mixed lymphocyte reactions can stimulate fibroblast proliferation in vitro. We demonstrated that interleukin-1 (IL-1), a lymphokine known to be produced by monocytes, was responsible for this effect. This was shown by comparison of thermocyte proliferative activation, the classical assay for IL-1, and fibroblast proliferative activation. The substances mediating these activities migrated together on gel filtration on sepacryl S-200, phenylsepharose chromatography, HPLC size exclusion chromatography and isoelectric focusing. Adding silica to purified monocyte monolayers stimulated the production of IL-1. The fibrosis observed in the lungs after silica inhalation may be explained by these observations. The general conclusion reached is that lymphokines and in particular IL-1 may have important effects on non-lymphoid tissues.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00226-01 LI

PERIOD COVERED

October 1, 1981-September 30, 1982

TITLE OF PROJECT (80 characters or less)

Rabbit Ig Allotypes: Structure, Organization and Regulated Expression of Ig Genes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Rose G. Mage	Sr. Investigator	LI/NIAID
OTHERS:	Leona C. Fitzmaurice	Research Expert	LI/NIAID
	Andrea Pavirani	Visiting Fellow	LI/NIAID
	Kenneth Bernstein	Medical Staff Fellow, Research Associate	LI/NIAID
	Nancy McCartney-Francis	Guest Worker	LI/NIAID

COOPERATING UNITS (if any) F. Mushinski, LCB/NCI; P. Reddy, LCMB/NCI; F. Blattner, Madison, Wisconsin; A. Chersi, Rome, Italy; P. Tucker, Dallas, Texas.

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NIAID/NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.5	3.5	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Our goals are to define in molecular terms the organization and regulated expression of rabbit immunoglobulin genes. Biologically active, physically intact mRNAs for rabbit heavy and light chains were prepared from rabbit spleens: cell free synthesis of allotype-specific γ,and light chains was demonstrated by immunoprecipitation. Clones carrying cDNAs encoding γ, κ and μ sequences were identified. An unexpectedly high nucleic acid sequence homology between the 3' untranslated (UT) sequence of b4 and b5 cDNAs was observed. A cDNA copy of μ mRNA, including 5' UT, leader, VH, D, J, Cμ, secreted tail piece and 3' UT is almost completely sequenced. The VH sequence contains codons for amino acids characteristic of the VHa2 allotype. cDNA probes have identified λ, κ, γ and μ mRNAs by Northern blot analyses. mRNAs that hybridize to κ and λ cDNA probes have also been detected in total RNAs prepared from cultured splenocytes. The high degree of homology of rabbit and mouse λ light chain sequence was confirmed by cross-hybridization of mouse λ1 cDNA to rabbit mRNA. Different mRNAs encoding one or more forms of mouse IgD have been identified and characterized. Our cloned and characterized cDNA probes allow us to study genomic DNA organization and regulated mRNA expression.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00227-01 LI

PERIOD COVERED
October 1, 1981 September 30, 1982

TITLE OF PROJECT (80 characters or less)
Biochemistry of Lymphocyte Activation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Myron J. Waxdal	Sr. Investigator	LI/NIAID
OTHERS:	Makoto Iwata	Visiting Fellow	LI/NIAID

COOPERATING UNITS (if any)

Fusao Hirata, LCM/NIMH

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Laboratory of Immunology

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INSTITUTE AND LOCATION
NIAID/NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.75	0.75	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) We have elucidated a series of biochemical steps in the activation of murine lymphocytes by mitogenic lectins. This biochemical pathway is apparently common to the activation of both T and B lymphocytes by a number of stimulants, including LPS, A23187, antiimmunoglobulin and soluble factors. This year's modest efforts conclude the first stages of the study, those assaying large numbers of lymphocytes. The next experiments are planned to analyze the early events in individual cells, through the use of fluorescent probes and flow microfluorometry. This refinement is necessary to establish the pathways followed by each cell type and the points at which intercellular interactions and communications (soluble factors) occur.

We have also found that an antibody directed against lipomodulin, a cell surface inhibitor of phospholipase A2 (one of the enzymes in the common activation pathway) blocks the con A stimulation of murine thymus cells. In addition, this antibody also inhibits, probably by a cytotoxic mechanism, the development of suppressor T cells.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00228-01 LI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)
Membrane Associated Glycoconjugates and Receptors as Differentiation Markers and Mediators of Cell-Cell Recognition and Communication.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. J. Waxdal	Sr. Investigator	LI/NIAID
OTHERS:	C. Kieda	Visiting Fellow	LI/NIAID
	C. Murre	Guest Worker	LI/NIAID

COOPERATING UNITS (if any)

Dr. M. Monsigny, CNRS, Orleans, France

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Laboratory of Immunology

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INSTITUTE AND LOCATION
NIAID/NIH, Bethesda, MD 20205

TOTAL MANYEARS: 2.75	PROFESSIONAL: 1.75	OTHER: 1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A nonagglutinating lectin, P-4, specifically recognizes surface glycoconjugates on human monocytes. The binding of this lectin can serve as a useful marker for human monocytes. The covalent coupling of Pa-4 to a solid support has provided an easy, reversible procedure for the removal of human monocytes from peripheral mononuclear cells.

Studies using neoglycoproteins (synthetic glycoproteins with selected carbohydrate moieties) have shown that subsets of human B and T cells can be defined by their surface carbohydrate receptors (specific endogenous lectins). Further, three neoglycoprotein probes have been synthesized which are bound by human null cells.

Human monocytes, and a subset of suppressor T cells, bind a complex carbohydrate isolated from pokeweed and can be specifically and completely removed from PBL by reversible agglutination with that carbohydrate. When placed in culture, these T cells produce a potent, soluble L-rhamnosespecific suppressor factor which shuts off immunoglobulin production by B cells.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00229-01 LI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies on Lymphocyte Differentiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Mark M. Davis	Staff Fellow	LI/NIAID
OTHERS:	David I. Cohen	Guest Worker	LI/NIAID
	Stephen M. Hedrick	Staff fellow	LI/NIAID
	Ellen A. Nielsen	Biologist	LI/NIAID
	William E. Paul	Chief	LI/NIAID

COOPERATING UNITS (if any)

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INSTITUTE AND LOCATION
NIAID/NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.1	2.1	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The object of this work is to provide a molecular framework with which to characterize B and T lymphocyte differentiation and function. This framework takes the form of recombinant DNA clones of B or T cell specific genes isolated through messenger RNA subtraction procedures (cDNA absorption, selected cDNA cloning) developed in this laboratory. During this past year we have concentrated our efforts in several regards: 1) estimating the differences in gene expression between B or T lymphomas and between tumors within the B or T lineage; 2) establishing libraries of B and T cell specific genes from selected tumors (selected cDNa libraries); 3) screening selected libraries for genes coding for cell surface and secreted proteins; 4) screening selected libraries for gene mapping to the X chromosome. In particular, we have isolated and partially characterized an X-linked gene family (TX-1) expressing multiple messenger RNA's in T helper hybridomas but not in other types of B and T cell tumors and hybridomas.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00259-01 LI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Ia Molecules and Immune Response Genes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	William E. Paul	Chief	LI/NIAID
OTHERS:	L.H. Glimcher	Research Associate	LI/NIAID
	Ira Green	Sr. Investigator	LI/NIAID
	Ronald H. Schwartz	Sr. Investigator	LI/NIAID
	Ellen Heber-Katz	Guest Worker	LI/NIAID
	Teruaki Hamano	Visiting Fellow	LMI/NIAID
	Kyung-Jin Kim	Visiting Associate	LMI/NIAID
	Richard Asofsky	Chief	LMI/NIAID

COOPERATING UNITS (if any)

Susan Sharrow, IB/NCI; David Sachs, IB/NCI

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Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.7	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Ia molecules appear to function as restriction elements in histocompatibility restricted antigen-recognition by T cells and as the products of specific immune response genes. In order to gain insight into the mechanisms through which Ia antigens express these functions, we have prepared a series of Ia mutants. To do this, we showed that mouse B lymphoma cell lines could act as antigen presenting cells (APC). B lymphoma-B cell hybridomas between the $H\text{-}2^{d/d}$ lymphoma line M12.4.1 and $H\text{-}2^{a/d}$ B cells were prepared and shown to have $I\text{-}A^{a}$ determinedAPC functions. A pair of monoclonal antibodies directed at distinct determinants on the $I\text{-}A^{a}$ Ia molecule were used to select mutants which expressed one but lacked the other determinant. These mutants lost the ability to present hen egg lysozyme but retained the ability to present a polymer of glutamic acid, alanine, or tyrosine to $H\text{-}2^{a}$ T cells. The structural basis of this change in presentation specificity is under study.

LABORATORY OF IMMUNOREGULATION
1982 Annual Report
Table of Contents

Z01-AI Project Number		Page
Summary		21-1
00210-02	Activation and Immunoregulation of Human Lymphocyte Function in Normal and Disease States - Fauci	21-12
00211-02	Study of Human Lymphocyte Subsets Employing Cloning and Hybridoma Technology - Fauci	21-13
00212-02	Study of the Immunopathogenic Features of Immune-Mediated Diseases - Fauci	21-14
00213-02	Clinical, Immunopathogenic, and Therapeutic Studies in the Spectrum of Vasculitis - Fauci	21-15

Summary Report
Laboratory of Immunoregulation
October 1, 1981 through September 30, 1982

Anthony S. Fauci, M.D.
Chief, Laboratory of Immunoregulation, NIAID
Deputy Clinical Director, NIAID

Development of A Model of Activation, Proliferation, and Differentiation of Human B Lymphocytes

Successful investigation of the sequence of events which take place from initial triggering of B cells through proliferation, through terminal differentiation is contingent upon systems which can dissociate these separate events from each other. We have developed an assay system in which a substantial proportion of the B cell repertoire can be activated with little or no proliferation or differentiation. Staphylococcus aureus Cowan I (SAC) has been shown to trigger human B cells to be activated in the absence of T cells and to manifest at least an initial, but self-limited, round of proliferation without terminal differentiation. Cells will not continue to proliferate unless appropriate B cell growth factors (BCGF) are added to cultures. Similar studies have been performed using anti-Ig as the initial signal to activate normal human B cells. Data accumulated from both these systems have led to a model of B cell activation in which the initial signal activates the B cells and induces the expression of receptors for BCGF. T-cell derived BCGF when added to cultures of these activated B cells induces (in the case of the anti-Ig triggering) or maintains (in the case of SAC triggering) B cell proliferation in the absence of differentiation. This capability of maintaining human B cells in a proliferative state without progression to terminal differentiation has served as the basis for studies in which B cells have been grown continuously in culture for up to 8 weeks without transformation. The dissociation between proliferation and differentiation of B cells and their sequential occurrence have been demonstrated in studies in which the cells which proliferate in the presence of BCGF will not differentiate, but will express receptors for a T-cell replacing factor (TRF) which should more accurately be called B-cell differentiation factor (BCDF) and which, in turn, induces terminal differentiation of the proliferating B cells to antibody-secreting cells. Thus, we have clearly demonstrated that a series of precise signals and distinct events occur from the initial activation of human B cells, through proliferation, up to and including terminal differentiation and antibody production. Appreciation of these mechanisms are critical to our understanding of normal human B cell physiology as well as the aberrancies operable in diseases characterized by hypo or hyperreactivity of B cell function (Muraguchi, Butler, Falkoff, Zhu, Fauci, LIR/NIAID).

Employing an in vitro system of soluble antigen-induced, antigen-specific responses of human peripheral blood B cells following in vivo immunization with antigens such as keyhole limpet hemocyanin (KLH) and tetanus toxoid (TT), which was developed in this laboratory in 1980-1981, studies were conducted over the past year which dissected out the various signals and mechanisms whereby human B cells were triggered to respond to specific antigen as well as delineated the precise relationship between the initial in vivo activation of the antigen-specific B cell repertoire and the subsequent in vitro responses to the antigen in question. Of critical importance to the success of these studies was the realization that the peripheral blood lymphocyte pool which is the most easily accessible and hence the most commonly studied lymphoid pool in normal and disease states in man undergoes a complex series of dynamic changes following perturbation of the system by introduction of antigen as with in vivo immunization.

Of particular interest and importance is the fact that we have rather strong evidence that induction of antigen-specific B cells which are present in the circulating B cell repertoire following primary in vivo immunization to secrete antibody is in fact independent of the in vitro binding to and direct triggering of B cells by the antigen. It is likely that the antigen-specific B cell is in a preactivated state following in vivo immunization and can be preferentially triggered to terminally differentiate by a nonspecific T cell factor. The specific antigen which is introduced into culture actually stimulates the antigen-specific T cell which in turn secretes such a factor. The remainder of the B cell repertoire apart from the antigen-specific B cells in question are not triggered by the T cell factor since they have not been "preactivated" in vivo by the immunizing antigen. In fact, under these circumstances specific antibody production is selectively triggered by T cell factor in the absence of in vitro antigen. Full appreciation of the physiology of B cell activation in man in whom studies are usually confined to the peripheral blood lymphocyte pool must take into account these considerations (Lane, Peters, Falkoff, Kehrl, Volkman, Fauci, LIR/NIAID).

Immunoregulation of Human B Lymphocyte Responses

We had previously demonstrated in our system of antigen-induced, antigen-specific B cell responses that while concentrations of KLH in the range of 50 ng/ml induced optimal specific antibody production, a concentration of 20 μg/ml induced specific unresponsiveness with regard to the production of anti-KLH antibody without suppression of total or polyclonal Ig production. We have now demonstrated that a complex series of events at the level of the B cell occur at these high antigen concentrations. B cells proliferate normally to these high antigen concentrations, and T cells with potent helper cell activity are induced by these concentrations. However, when antigen-reactive B cells are exposed to these high concentrations of antigen together with a differentiation signal either from a triggered T cell or a T cell factor, blockade of terminal differentiation occurs. This antigen-induced reversible induction of unresponsiveness directly at the B cell level may be an important mechanism of modulation of B cell function in normal and disease states (Lane, Volkman, Fauci, LIR/NIAID).

We further delineated the complex modulation of this antigen-specific human B cell model by immunoregulatory T cell subsets. We examined the T cell subsets involved in antigen (KLH)-induced compared to mitogen (PWM)-induced help by means of limiting dilution analysis and differential radiosensitivity and demonstrated that specific and nonspecific helper T cell functions are delivered by distinct subpopulations of T cells with different precursor frequencies and different radiosensitivities. In other studies, T cells were separated into T4+ and T8+ subpopulations using monoclonal antibodies, and their modulation of antibody synthesis was studied. T4+ cells functioned as helper cells in both antigen-driven and PWM-driven cultures in a dose-dependent manner. T8+ cells suppressed the PWM-driven antigen-specific and polyclonal responses, but they did not suppress the antigen-driven responses. The reason for this lack of suppression of antigen-specific B cell responses by T8+ cells was the fact that antigen was incapable of triggering the T4+ inducers of T8+ suppressor cells, whereas PWM was a sufficient signal to trigger the T4+ inducer cells. These studies demonstrate a clearcut dichotomy between antigen-induced and mitogen-induced human B cell responses with regard to the triggering of immunoregulatory T cell circuits (Peters, Lane, Fauci, LIR/NIAID).

For the first time in a human system, we demonstrated the role of the monocyte in the Ia-associated presentation of antigen to T cells and the genetic restriction of monocyte-T cell interactions in the induction of antigen-specific T-cell dependent B cell responses. Monocytes could present antigen to antigen-specific T cells only in the context of compatibility at the HLA-DR locus. Using cloned antigen-specific T cells (discussed below), we unambiguously demonstrated that DR compatibility between the monocyte and T cell is required at only 1 of the 2 DR haplotypes depending on the clone in question. In addition, we demonstrated the complex and multifaceted effects of monocyte-derived factor interleukin 1 (IL 1) on the regulation of human B cell reactivity. Furthermore, we demonstrated the complex positive and negative influences which allogeneic effects exert on antigen-specific B cell responses as opposed to polyclonal responses (Volkman, Gerrard, Lane, Fauci, LIR/NIAID).

Finally, it would be of particular clinical relevance to be able to eliminate specifically and exclusively antigen-reactive B cells. This would have obvious implications in the elimination of hyperreactive B cells in certain hypersensitivity and autoimmune states. One of the methods of specifically eliminating such B cell subsets is to poison the cells by a technique which would damage only the antigen-reactive B cells in question. In this regard, the powerful cytotoxic substance ricin was conjugated to a given antigen, in this case either KLH or TT. The ricin conjugated carrier was then introduced into a suspension of cells containing the antigen-reactive B cells. The antigen bound to the B cells in question and thus brought the ricin in close enough proximity to damage only the B cell which had bound the antigen. In this respect, only the antigen-specific B cells were eliminated by the cytotoxic ricin. This is the first demonstration in the human or animal model of the selective elimination of antigen-specific B cells by virtue of the antigen specificity of the surface membrane Ig of the B cell while leaving the remainder of the B cell repertoire intact (Volkman, Fauci, LIR/NIAID; Ahmad, Neville, NIMH).

Pharmacologic Modulation of Human Immune Responses

Corticosteroids are widely used in clinical medicine in the treatment of inflammatory and/or immune-mediated diseases. This laboratory has been involved over the past several years in the delineation of the in vivo and in vitro effects of corticosteroids on human immune function. Over the past years, these studies have centered on the effects of corticosteroids on the activation and immunoregulation of human B cell function. A number of interesting observations were made including the selective suppression of chronically administered corticosteroids on human B cell function by a number of complex mechanisms including the selective enrichment in the circulation for suppressor T cells by the predominant depletion of helper T cells. This depletion was effected by a shift in the population of helper T cells to extravascular lymphoid compartments. Additional studies were aimed at determining the mechanisms of the phenomenon which is sometimes observed clinically whereby corticosteroids actually potentiate antibody responses under certain circumstances.

A number of studies were performed on the precise mechanisms of action of cytotoxic agents on the activation and immunoregulation of human lymphocyte function associated with our study of a number of patient populations treated with these agents. It was found that cyclophosphamide had a highly selective effect on human B cell function with relative sparing of the T cell subsets at dosages of drug used in patients with immune-mediated diseases. Of particular note was the fact that the equivalent dose regimens of azathioprine therapy had no similar suppressive effect on the B cell function of our patient population. This observation is of particular relevance given the fact that cyclophosphamide has been found to be significantly more effective than azathioprine in the treatment of B cell mediated diseases. This observation lends a scientific explanation to a commonly observed clinical phenomenon (Cupps, Fauci, LIR/NIAID).

Development of Human B Cell Hybrids From Human Peripheral Blood Secreting Monoclonal Antibody Against Preselected Antigenic Specificities

Utilizing the nonsecreting, HAT-sensitive mouse myeloma line SP-1 and the peripheral blood mononuclear cells from individuals recently immunized to the soluble protein antigens KLH, TT, or to pneumococcal polysaccharide (PPS), we have produced stable heterohybridomas which secrete human monoclonal antibodies directed towards the immunizing antigen. The cell lines esbablished have been maintained in continuous culture for up to 18 months and secrete from 10 to 30 μg/ml of monoclonal human antibody. The ability to choose an antigen, immunize a human subject to that antigen, and then use the peripheral blood lymphocytes from that subject to produce a hybrid cell line secreting antigen-specific human Ig is of potential great value in a wide variety of investigative, diagnostic, and therapeutic endeavors.

Because of the potentially extremely important clinical implications of being able to readily produce B cell hybrids from the easily accessible lymphoid compartment of human peripheral blood, we developed a protocol for optimizing the immunization of donors, for delineating the kinetics of the availability of fusable B cells in the peripheral blood, and for enhancing the in vitro conditions for fusing.

The most critical component of the protocol was obtaining the peripheral blood lymphocytes for fusing at a time when the desired antigen-specific B cells were present in the circulation and thus readily accessible. This point cannot be overemphasized. In our studies, normal volunteers were booster immunized with TT, and their blood drawn at various intervals ranging from 0-15 days following booster immunization. The peripheral blood lymphocytes obtained at these times were then studied for their ability to spontaneously secrete anti-TT antibodies, or to form heterohybridomas secreting anti-TT antibodies. At approximately 7 days following booster immunization, the number of cells in the peripheral blood spontaneously secreting anti-TT antibody as well as the ability of the cells in the peripheral blood to form heterohybridomas secreting anti-TT antibody are both at a maximum and demonstrate a kinetic pattern similar to that seen following KLH or PPS immunization. By 15 days following booster, both parameters have returned to baseline. Thus, failure to obtain the peripheral blood for fusion at an appropriate time following booster immunization will result in a drastic reduction in the yield of antigen-specific hybridomas (Lane, Butler, Kehrl, Zhu, Fauci, LIR/NIAID).

Development of Human T-T Cell Hybridomas from Peripheral Blood T Cells

In studies based on the hybridization techniques outlined above, normal human peripheral blood T cells were fused with the HAT-sensitive human T-leukemic cell line, CEM-6. Supernatants from the resulting hybrid cells were tested for the ability to maintain proliferation of normal human B cells in our assay system for human BCGF. Hybrids demonstrating BCGF activity were cloned by limiting diluton. One hybrid clone, $2B_{11}$, continued to support proliferation of B cells in both long-term cultures and 6-d assays at a level significantly above that seen with conventionally produced growth factors. No IL2 activity was found in the supernatant from hybrid $2B_{11}$. The hybridoma supernatant was fractionated by gel filtration, and maximal proliferation of B cells was supported by the 18-20,000 daltons protein fraction. Thus, a human T-T cell hybridoma which has BCGF activity in the absence of any demonstrable IL2 activity has been developed. In additional studies using similar methodologies, we have developed a panel of human T-T cell hybrids which individually secrete one of a number of immunoregulatory molecules such as helper, amplifier, and suppressor factors. Human T-T cell hybridomas secreting discrete immunoregulatory factors should prove to be powerful tools in dissecting the mechanisms of immunoregulation of human lymphocyte function (Butler, Muraguchi, Fauci, LIR/NIAID).

Cloning of Antigen-Specific T Cells from Human Peripheral Blood

In 1980-1981, we developed the first soluble antigen-specific human T cell clones. Over the past year, we have extended these studies and have developed more efficient cloning methodologies which has allowed us to produce a panel of antigen-specific clones. We have demonstrated the feasibility of immunizing individuals with a variety of soluble protein antigens such as KLH and TT and developing T cell clones which are specific for these antigens. Using this system, we have demonstrated that such clones proliferate only in response to the antigen in question, they recognize antigen only in association with antigen-presenting cells under conditions of strict HLA-DR restriction at 1 of the 2 DR haplotypes. In addition, clones were obtained which express either the suppressor OKT8 or helper OKT4 phenotype and manifest either suppressor or helper function respectively. Availability of these cloned populations of antigen-specific immunoregulatory T cells should be of extraordinary value in dissecting out the mechanisms of immunoregulation of human lymphocyte function, in the delineation of MHC restriction in human immune responses, and in endeavors towards defining the T cell receptor for antigen (Volkman, Fauci, LIR/NIAID); Matis, Medicine Branch, NCI).

Identification and In Vitro Correction of a Natural Killer (NK) Cell Defect in the Chediak-Higashi Syndrome (CHS)

In 1980-1981, we made the extremely interesting observation that patients with CHS have a selective and isolated deficiency in NK cell activity. This was the first description in man of a primary immunodeficiency disease with an isolated defect in NK cells. This has potentially important clinical implications since CHS patients have a propensity to develop lymphoid malignancies. Over the past year, we demonstrated that the defect was at the level of the "active" NK cell in that patients with CHS have normal numbers of NK cells and normal percentages of target binding NK cells. The defect was demonstrated to be a functional defect in that NK cells which bind to target cells and should thus be "active" NK fail to subsequently kill the target. We then demonstrated by in vitro studies that cyclic guanosine monophosphate (cGMP) or inducers of cGMP could correct the defect in NK function in CHS. These latter studies underscore the role of cyclic nucleotides in the regulation of NK function and raises the possibility of a therapeutic modality in patients with defects in NK function (Katz, Univ. of Fla.; Fauci, LIR/NIAID).

Studies in the Kaposi Sarcoma - Opportunistic Infection Syndrome

Over the past year, the LIR has been involved in studying the immunopathophysiologic components of this fascinating new syndrome. We undertook intensive examination of various immunological parameters in patients with this syndrome and attempted to define the extent and mechanisms of the profound immune defect as well as the precise nature of the dichotomy between the T cell defect and the apparent sparing of B cell responses.

The common denominator of the pathophysiology of this syndrome is a profound immunosuppression with an extraordinary selective diminution of the T4 or Leu 3 inducer/helper subset of T cells. Patients have marked suppression of cell-mediated immune function with apparent sparing of humoral immunity since they often manifest hypergammaglobulinemia. Of particular interest is the fact that although they have hypergammaglobulinemia and their B cells function relatively normally in vitro, provided helper T cells are present, we have been unable to induce in vivo antibody production in these individuals by immunization with a new antigen such as KLH, nor have we been able to demonstrate in vitro responses, either T cell or B cell, to the antigen following in vivo immunization. Given the high likelihood that a significant proportion of the gammaglobulin of the hypergammaglobulinemic state of these individuals resulted from T-cell dependent B cell responses, this finding of severe deficiency of B cell responses to a T-cell dependent antigen in these patients attests to the complexity of the immunoregulatory abnormalities of this syndrome (Fauci, Lane, LIR/NIAID; Masur, MICV/CC).

Studies in the Idiopathic Hypereosinophilic Syndrome (HES)

A broad range of clinical and pathophysiologic studies were performed in a large number of patients with HES. The precise mechanisms of organ system damage and dysfunction were delineated predominantly with regard to the endocardiomyopathy of the HES. The major basic protein of the eosinophil was demonstrated to be directly toxic to endothelial cells and likely accounts for the first insult to the endocardium. This was followed by a laying down of platelet thrombi with subsequent mural thrombosis and endocardial fibrosis leading to the restrictive cardiomyopathy. Although a portion of these studies were performed in an in vitro system, they are the first to strongly suggest the role of an eosinophil product in the direct damage to human tissue in the HES. In addition, in vitro stem cell studies delineated the heterogeneous levels of abnormalities in patients with HES ranging from primary aberrancies of eosinophil production to the regulation of eosinophilopoiesis. Furthermore, predictive parameters of the heart disease in HES as well as specific genetic profiles were described. Aggressive medical and surgical approaches to the heart disease as well as chemotherapeutic regimens aimed at the underlying eosinophilia have led to a dramatic improvement in prognosis of the HES. The study of these patients has allowed the possibility of studying several of the functional capabilities of the eosinophil ranging from those related to their enzyme content to others relating to the cytotoxic and other functional capabilities of the cells. (Harley, Fauci, LIR/NIAID; Gleich, Mayo Clinic; Bjornson, Tufts Medical Center; Gralnick, Hematology Service/CC).

Mechanisms of Abnormal Cellular Activation and Immunoregulation in Immune-Mediated Diseases

Utilizing the above-mentioned systems of specific and nonspecific triggering and immunoregulation of human B cells as well as the delineation of immunoregulatory T cell and monocyte subsets, a series of studies were performed to delineate the abnormalities of immune function in a number of immune-mediated diseases. It was demonstrated that patients with Hashimoto's thyroiditis had abnormally high numbers of circulating thyroglobulin (Tg)-reactive B cells as well as abnormally activated T cells. In addition, they were capable of being triggered by specific and/or nonspecific stimuli to secrete anti-Tg antibody. This system will prove invaluable in examining the mechanisms of aberrant immune function in this disease. In addition, this laboratory has been involved in studies of abnormal cellular activation and immunoregulation in Sjogren's syndrome as well as in systemic lupus erythematosus for a number of years. Over the past year, a number of studies further delineating the mechanisms of hyperreactive B cells as well as deficiency of immunoregulatory T cells were carried out.

We have studied a number of T cell neoplasms and have correlated their phenotypic profile with their in vitro functional capability. In this regard, we have made certain extremely interesting and potentially important observations regarding the full spectrum of pathophysiologic manifestations in patients with T cell neoplasms. For example, we have demonstrated that patients with fulminant erythrophagocytosis resembling histiocytic medullary reticulosis in fact have an underlying T cell neoplasm which secretes a macrophage-activating factor responsible for inducing extensive erythrophagocytosis in vivo. In addition, we are currently investigating the relationship between the profound eosinophilia in certain patients with T cell neoplasms and the secretion by the malignant neoplasm of an eosinophilopoietic and/or eosinophilochemotactic factor. Such studies will shed light on mechanisms of disease expression as well as serve as an excellent model of monoclonal functional T cells (Fauci, Lane, LIR/NIAID; Jaffe, LP/NCI).

Clinical, Immunopathogenic, and Therapeutic Studies in the Spectrum of Vasculitis

The LIR is now prospectively studying the largest group of patients with the vasculitic syndromes of any center in the world. Clinical, pathophysiologic, immunopathogenic, and therapeutic results obtained over the past 14 years have allowed us to design a revised categorization scheme for the vasculitides which has now reached world wide acceptance. We have instituted aggressive chemotherapeutic regimens in several formerly universally fatal diseases such as Wegener's granulomatosis and have induced cures in 90% or more of these patients. We have now applied these approaches with remarkable success to other of the vasculitic syndromes including systemic vasculitis of the polyarteritis nodosa group, isolated central nervous system vasculitis, Takayasu's arteritis, recurrent panniculitis with vasculitis, and the vasculitis of Sjogren's syndrome. Our categorization schemes and the therapeutic protocols designed by us are now widely accepted and are being utilized by medical centers throughout the world. We have now established linkages between several of these diseases and various immune response genes as indicated by HLA-DR haplotype associations. These patient populations have been utilized to precisely delineate aberrations of activation and immunoregulation of lymphoid cell function in man and have served as excellent models for the concomitant study of the normal human immune system. In addition, the precise effects of various therapeutic regimens, particularly corticosteroids and cytotoxic agents, on human lymphoid cell activation and immunoregulation have been described (Fauci, Cupps, Volkman, Lane, LIR/NIAID).

Administrative, Organization, and Other Changes

The Laboratory of Immunoregulation (LIR) was established towards the end of 1980 and is now almost 2 years old. The theme of the laboratory is to study in a concentrated manner the mechanisms of activation and immunoregulation of human immunocompetent cells, particularly B lymphocytes, in normal individuals and in a variety of disease states characterized by abnormalities of immune function. In addition, the LIR continues to conduct the bulk of the clinical studies carried out in the NIAID intramural component of the Clinical Center.

When the LIR was established, the plan for personnel was to develop predominantly, but not necessarily exclusively, from the existing Clinical Associate and Fellow pool a group of individuals who will be staying on for variable periods of time both to maintain continuity in ongoing studies as well as to serve as a source of individuals who might potentially ultimately assume senior positions in the laboratory. In this regard, Drs. David Volkman and Thomas Cupps have remained in the laboratory for an additional year following the termination of their Clinical Associateship. This has proven to be a productive year for both of them and for the LIR. Starting July 1, 1982, Dr. Cupps will continue for an additional year; Drs. Volkman and H. Clifford Lane will continue in the Public Health Service (PHS) with the ultimate intent to get into a tenure tract. Dr. Reuben Falkoff will also be remaining on for an additional year starting July 1, 1982; however, because of budget constraints, he will leave the PHS and be reinstated as a Medical Staff Fellow. Dr. John Harley left the laboratory in June 1982 at the termination of his Clinical Associateship to join Dr. Robert Good at the Oklahoma Research Center at the University of Oklahoma. Drs. Lauren Charous and Joseph Margolick began their second year of Medical Staff Fellowship after completing their clinical year. Drs. John Kehrl and Joseph Butler began their third year of their Clinical Associateship, and we intend to keep them on for an additional year in much the same manner as was done with the Clinical Associates listed above. Dr. Atsushi Muraguchi joined us from Osaka, Japan, in October 1981 as a Visiting Fellow and will continue on for a second year. Dr. Li-Ping Zhu has been with us as a Guest Worker from Peking and will continue for an additional year. Dr. Theresa Gerrard is a Staff Fellow who will also continue for her third year. Dr. Marion Peters is a Visiting Fellow from Australia who will finish her fellowship in May 1983 and will leave the group. Because of the substantial load of manuscripts which the LIR has generated over the past year, Mrs. Ann London has been added to the staff as an Editorial Assistant.

Renovations on the 11th Floor B Wing of the Clinical Center will be completed in August 1982, and members of the LIR who are occupying space in modules in various corridors will now be consolidated on the B Wing such that the laboratory will now be contained entirely within the 11th Floor B Wing.

Honors, Awards, and Scientific Recognition

Over the past year, members of the Laboratory of Immunoregulation, predominantly in the person of Dr. Fauci, Chief, LIR, have received a number of awards and honors. First, Dr. Fauci has served on a number of committees of scientific note. He is currently the Program Chairman for the national meeting of the American Association of Immunologists. He is serving on the Committee on Clinical Immunology and Immunopathology of the American Association of Immunologists. Dr. Fauci is also a representative of The American Association of Immunologists to the Examinations Committee of the American Board of Allergy and Immunology. In addition, Dr. Fauci is on the post-graduate education committee of the American Academy of Allergy and is a member of the prestigious Section of Physiology in Clinical Science of the American Physiology Society. It is of note that he is the only immunologist on this well-known section on Physiology in Clinical Sciences which is organized and run by the American Physiology Society. In addition, he is a member of the subcommittee on the classification of vasculitis of the American Rheumatism Association and is on the ad hoc committee of the National Foundation for Infectious Diseases.

Dr. Fauci serves on a number of editorial boards of journals concerned with the areas of immunology, allergy, and infectious diseases. He is the Section Head of the Editorial Board of the Clinical Immunology Section of The Journal of Immunology. In addition, he is the Associate Editor in charge of Allergy and Immunology of the American Journal of Medicine. Dr. Fauci also serves on the editorial boards of The Journal of Clinical Investigation, The Journal of Infectious Diseases, The Annals of Allergy, The Journal of Immunopharmacology, The Journal of Clinical Immunology, the new series entitled Clinics in Immunology and Allergy, and the new journal EOS. In addition, Dr. Fauci in collaboration with Dr. John I. Gallin of the LCI, NIAID, has created and is the co-editor of the newly established series Advances in Host Defense Mechanisms. Dr. Fauci has also edited the book entitled Human B Lymphocyte Function: Activation and Immunoregulation. Finally, together with Dr. Lawrence Lichtenstein, Dr. Fauci is editing a new textbook entitled Current Therapy in Allergy and Immunology. Over the past year, Drs. Cupps and Fauci have written the highly regarded book entitled The Vasculitides. Dr. Fauci has also contributed invited chapters on a number of subjects for most of the major textbooks of medicine as well as subspecialty textbooks in Immunology, Allergy, and Infectious Diseases.

As part of the recognition for scientific accomplishments, clinical investigators may be asked to visit outside institutions and serve for periods of from two to three days as visiting professor within a given institution. In this regard, Dr. Fauci had been asked and did serve as visiting professor at several major institutions during the year. Among these were his visiting professorships at the University of Michigan Medical Center as well as the Paul B. Beeson Visiting Professorship at Yale University School of Medicine.

In addition, Dr. Fauci was asked to give several major or named lectureships during the year. He delivered the prestigious Paul B. Beeson Lecture at Yale University School of Medicine. He was one of the major invited symposium speakers at the annual meeting of the Infectious Diseases Society of America. He was the plenary speaker at the annual meeting of the Midwest Society for Pediatric Research. He was the Chairman and Symposium Speaker at the Scientific Workshop of the annual meeting of the American Rheumatism

Association. He was an invited symposium speaker at the National Meeting of the American Association of Immunologists. Finally, the work of the laboratory and its members were recognized in that 6 papers from the LIR were presented at the highly competitive program of the National Clinical Meeting of the AAP/ASCI/AFCR.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AI-00210-02 LIR

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Activation and Immunoregulation of Human Lymphocyte Function in Normal and Disease States

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator: Anthony S. Fauci, M.D., Chief, LIR/NIAID

Other Investigators:
H. C. Lane, M.D., Clinical Associate, LIR/NIAID
D. Volkman, M.D., Ph.D., Medical Officer, LIR/NIAID
T. Cupps, M.D., Medical Officer, LIR/NIAID
R. Falkoff, M.D., Ph.D., Clinical Associate, LIR/NIAID
J. Harley, M.D., Ph.D., Clinical Associate, LIR/NIAID
J. Butler, M.D., Clinical Associate, LIR/NIAID
J. Kehrl, M.D., Clinical Associate, LIR/NIAID
M. Peters, M.D., Visiting Fellow, LIR/NIAID
T. Gerrard, Ph.D., Staff Fellow, LIR/NIAID
A. Muraguchi, M.D., Visiting Fellow, LIR/NIAID
Liping Zhu, M.D., Guest Worker, LIR/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Immunoregulation, NIAID

SECTION

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6	4	2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) A model of human B cell activation, proliferation and terminal differentiation was established in which the spectrum of specific and non-specific activation signals as well as various growth and differentiation factors were precisely delineated. Immunoregulatory T cell and monocyte subsets and the diverse mechanisms of their modulation of B cell responses were delineated. Radiation sensitivity and precursor frequency studies of immunoregulatory T cells as well as limiting dilution analyses of antigen-specific B cells were performed. The phenomenon of high antigen concentration blockade of B cell differentiation in the face of normal B and T cell proliferation was described. The relationship between the primary activation of B cells by antigen and the subsequent antigen-independent driving of these cells towards terminal differentiation by non-specific soluble factors was studied. The genetic restriction of monocyte-T cell interactions in the induction of T cell dependent B cell responses as well as the role of Ia and certain cell surface activation molecules in the antigen-induced activation of B cells were described. Selective elimination of antigen-reactive B cell clones by antigen-toxin conjugates was demonstrated. The complex mechanisms of modulation of human B cell responses by corticosteroids and cytotoxic agents were defined.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AI-00211-02 LIR

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Study of Human Lymphocyte Subsets Employing Cloning and Hybridoma Technology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator: Anthony S. Fauci, M.D., Chief, LIR/NIAID

Other Investigators:

David Volkman, M.D., Ph.D., Medical Officer, LIR/NIAID
H. Clifford Lane, M.D., Clinical Associate, LIR/NIAID
Joseph Butler, M.D., Clinical Associate, LIR/NIAID
Liping Zhu, M.D., Guest Worker, LIR/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland

TOTAL MANYEARS: 4	PROFESSIONAL: 3	OTHER: 1

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Major advances in the development of B cell hybridomas secreting monoclonal human antibodies were realized. Following in vivo immunization of normal humans with various preselected antigens such as KLH, tetanus toxoid, and pneumococcal polysaccharide, peripheral blood B cells were fused with a HAT sensitive mouse myeloma line to yield extremely stable (greater than 18 months) heterohybrids secreting large amounts (10-30 ug/ml) of monoclonal human antibody. Precise conditions for optimizing the yield of hybrids from human peripheral blood were delineated. T-T cell hybrids secreting immunoregulatory factors were established by the fusion of human blood T cells and a HAT sensitive human T cell leukemia line. Of note was the development of hybrids which secrete B cell growth factors devoid of T cell growth factor or B cell differentiation factor activity thus providing a source of purified factor for the continuous growth of B cell lines. Improved methodologies for the cloning of antigen-specific human T cells from peripheral blood were developed and resulted in the production of individual clones specific for KLH, tetanus toxoid or diphtheria toxoid. Both functional helper and suppressor clones were developed. These clones were antigen-specific and MHC restricted in the recognition of soluble antigen in the context of DR or MB antigen bearing presenting cells.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00212-02 LIR

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Study of the Immunopathogenic Features of Immune Mediated Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator: Anthony S. Fauci, M.D., Chief, LIR/NIAID

Other Investigators:

Thomas R. Cupps, M.D., Medical Officer, LIR/NIAID
H. Clifford Lane, M.D., Clinical Associate, LIR/NIAID
John B. Harley, M.D., Clinical Associate, LIR/NIAID
Gerald Gleich, M.D., Mayo Clinic
Paul Katz, M.D., University of Florida Medical Center

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland

TOTAL MANYEARS: 3	PROFESSIONAL: 2	OTHER: 1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Immunopathogenic studies were performed on patients with a diversity of immune mediated diseases. An isolated defect in NK cell activity was described in patients with the Chediak-Higashi syndrome constituting the first description of a primary human immunodeficiency disease with an isolated defect in NK cells. This selective defect was corrected in vitro by inducers of cAMP thus providing a basis for potential correction of this defect in vivo. Patients with the Kaposi sarcoma-opportunistic infection syndrome seen predominantly among male homosexuals were studied. A profound and selective defect in the T4 inducer subset of T cells was demonstrated together with a defect in the ability to respond in vivo and in vitro to T cell dependent B cell stimulation despite the presence of hypergammaglobulinemia in vivo. A large number of patients with the idiopathic hypereosinophilic syndrome (HES) were studied. Mechanisms of organ system damage and dysfunction were delineated predominantly with regard to the role of the eosinophil in the endocardial damage characteristic of HES. Mechanisms of aberrant B cell activation and immunoregulation were delineated in several autoimmune diseases of both the organ specific (Hashimoto's thyroiditis) as well as the organ non-specific (systemic lupus erythematosus and Sjogren's syndrome) type.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AI-00213-02 LIR

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Clinical, Immunopathogenic and Therapeutic studies in the Spectrum of Vasculitis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator: Anthony S. Fauci, M.D., Chief, LIR/NIAID

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COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland

TOTAL MANYEARS: 2	PROFESSIONAL: 2	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The LIR is prospectively studying the largest group of patients with the vasculitic syndromes in the world. Clinical, pathophysiologic, immunopathogenic and therapeutic results obtained over the past 14 years have allowed us to design a revised categorization scheme for the vasculitides which has now reached world wide acceptance. We have instituted aggressive chemotherapeutic regimens in several, formerly universally fatal diseases such as Wegener's granulomatosis and have induced cures in 90% or more of these patients. We have now applied these approaches with remarkable success to other of the vasculitic syndromes including systemic vasculitis of the polyarteritis nodosa group, lymphomatoid granulomatosis, isolated CNS vasculitis, Takayasu's arteritis, and the acute vasculitis of Sjogren's syndrome. We have identified a remarkable 100% association of B cell alloantigen MB 3 and Takayasu's arteritis in Americans. These patient populations have been utilized to precisely delineate aberrations of lymphocyte activation and immunoregulation and have served as excellent models for the study of normal human immune function. In addition, the precise effects of various therapeutic regimens, particularly corticosteroids and cytotoxic agents, on human lymphoid cell activation and immunoregulation have been described.

I N D E X

LABORATORY OF INFECTIOUS DISEASES

Z01 AI LID Project Number		Page
Summary Statement		22-1

Hepatitis

00308-01	*In Vitro* Studies of Hepatitis A Virus - Daemer	22-27
00309-01	The Biology of Hepatitis A Virus - Feinstone	22-28
00321-01	Molecular Virology of Hepatitis A Virus (HAV) - Ticehurst	22-29
00310-01	Hybridoma Antibodies to Pathogenic Viruses - Feinstone	22-30
00311-01	Search for New Hepatitis Agents - Purcell	22-31
00312-01	Clinical Studies of Hepatitis B Vaccines - Ticehurst	22-32
00313-01	Molecular Biology of Hepatocellular Carcinoma - Daemer	22-33
00314-01	Woodchuck Hepatitis Virus - Purcell	22-34
00315-01	Search for New Hepadnaviruses - Ticehurst	22-35
00316-01	The Delta Agent - Purcell	22-36
00317-01	Biology of Non-A, Non-B Hepatitis Agents - Feinstone	22-37
00318-01	Molecular Biology of Non-A, Non-B Hepatitis Agents - Purcell	22-38
00319-01	New Approaches to the *In Vitro* Propagation of Non-Cultivatable Agents - Daemer	22-39
00320-01	Parvovirus-Like Agents and Hematological Disorders - Purcell	22-40

RS Viruses

00322-01	Molecular Biology of Respiratory Syncytial Virus - Venkatesan	22-41

Z01 A1 LID

00323-01 Pneumovirus and Paramyxovirus Surface Glycoproteins - Venkatesan 22-42

00344-01 Immunologic Characterization of Respiratory Syncytial Viruses - Prince 22-43

00345-01 Immunity to RS Virus - Prince 22-44

Influenza A Virus

00324-01 Laboratory Studies of Myxoviruses - Murphy 22-45

00325-01 Study of Respiratory Viruses in Primates - Murphy 22-46

00326-01 Study of Respiratory Viruses in Volunteers - Murphy 22-47

00327-01 Characterization of Influenza A Viruses Using Monoclonal Antibodies - van Wyke 22-48

Molecular Biology

00328-01 Requirements for Cell Surface Expression of Influenza A Virus Hemagglutinin from Cloned Full-Length DNA - Sveda 22-49

00329-01 Studies of an Influenza A Virus Neuraminidase Gene Using Recombinant DNA Techniques - Markoff 22-50

00330-01 Cloning, Expression and Functional Domains of the Influenza Virus Gene Coding for Nucleoprotein - Lin 22-51

00331-01 Transcriptional Control of Influenza A Virus: Synthesis of Spliced and Unspliced mRNAs - Lai 22-52

00332-01 Allele Replacement of Influenza Gene Segments Using CLoned DNA Sequences - Lai 22-53

Viral Gastroenteritis

00343-01 The Role of Norwalk-like 27 nm Virus Particles in Viral Gastroenteritis - Greenberg 22-54

00333-01 A Longitudinal Study of Viral Gastroenteritis in Infants and Young Children and Other Epidemiological Studies - Kapikian 22-56

00334-01 Development of Monoclonal Antibodies to Proteins of Rotavirus - Greenberg 22-57

00335-01 Development and Phenotypic Analysis of Rotavirus Reassortants - Greenberg 22-58

Z01 AI LID

00336-01 Identification and Characterization of Rotavirus Gene Products - Kalica 22-59

00337-01 Rotaviral Proteins - Kalica 22-60

00346-01 Genetic Diversity of Rotaviruses Studied by RNA-RNA Hybridization - Flores 22-61

00338-01 Characterization of the Rotavirus Genome by Recombinant DNA Techniques - Flores 22-62

00339-01 Cultivation and Serotypic Characterization of Human and Animal Rotaviruses - Wyatt 22-63

00340-01 Experimental Studies in Animals with Various Rotaviruses - Wyatt 22-64

00341-01 Studies of Rotaviruses in Volunteers - Wyatt 22-65

00342-01 Studies of Gastroenteritis Viruses by Electron Microscopy - Kapikian 22-66

SUMMARY STATEMENT

Annual Report

Laboratory of Infectious Diseases
National Institute of Allergy and Infectious Diseases
October 1, 1981 to September 30, 1982

HEPATITIS

Hepatitis A - Hepatitis A virus (HAV), originally first visualized by LID scientists, is responsible for most epidemics of short incubation hepatitis. The virus is a picornavirus that most closely resembles the enteroviruses. This agent was only recently propagated in tissue culture for the first time by scientists at Merck, Sharp and Dohme. Several laboratories, including the Hepatitis Viruses Section of LID, have succeeded in confirming this finding. At present 20 serial passages in primary African green monkey cells have been achieved in LID (Daemer, Feinstone, Purcell). Most of the antigen remains cell-associated and initially 8 weeks or longer were required to demonstrate evidence of viral replication. Following serial passage, this interval decreased to 1-4 weeks. With passage, the level of viral replication increased from 10^5 at the 10th passage to 10^8 at the 16th passage. These findings indicate that a mutant that is adapted to growth in cell culture had been selected.

At the tenth passage, virus was inoculated into chimpanzees and was observed to be highly attenuated. There was essentially no disease as judged by serum alanine amino transferase (ALT) level, virus could not be detected in the liver by immunofluorescence, and only one stool sample contained hepatitis A antigen as detected by RIA. However, chimpanzees inoculated with as little as one chimpanzee infectious dose of the attenuated virus produced an excellent immune response. Further investigations of this attenuated strain of HAV are in progress to determine if it might be useful as a live vaccine. The tissue culture-adapted HAV mutant is being patented and two pharmaceutical manufacturers have expressed an interest in its potential as a strain for use in a live virus vaccine (Feinstone, Purcell).

Additional studies with HAV now under way in LID include attempts at molecular cloning of genomic sequences in *E. coli* and the production of monoclonal antibodies directed against the virus (Ticehurst, Feinstone).

On occasion, epidemics of short incubation, water-borne hepatitis occur that are not caused by HAV, although their epidemiology closely resembles that of HAV. Three such outbreaks that occurred in India are being studied in collaboration with Dr. Khorshed Pavri. These outbreaks appear to be caused by an agent serologically distinct from currently recognized HAV strains. Attempts to transmit an agent from clinical specimens from these outbreaks have been only partially successful. Transient low-level liver enzyme elevations and histopathologic changes consistent with hepatitis in liver biopsies have been observed in some animals, but this has not been uniform, and attempts to serially transmit an agent in chimpanzees and marmosets have also produced irregular results. Characteristic histopathologic changes distinct from those seen in type A hepatitis, type B hepatitis and non-A, non-B hepatitis have been reported by Dr. Hans Popper (Mt. Sinai, New York) (Purcell).

Hepatitis B - Hepatitis B virus (HBV) is a unique 42nm virus of complex structure that contains a double-stranded circular DNA with a single-stranded gap spanning 20-50 percent of the genome. It is the first recognized member of what is now a small group of viruses unofficially designated the "hepadna" viruses that also includes the woodchuck virus, the Pekin duck virus and the Beechey squirrel virus. HBV is responsible for considerable morbidity and mortality, accounting for 30-50 percent of clinical hepatitis diagnosed in the U.S. and most developed countries. However, it is in Asia and Africa that the virus has the greatest impact. It is the cause of chronic infection, usually associated with hepatitis, in approximately five percent of the world's population, and such chronic infection may lead to death from chronic hepatitis, cirrhosis or hepatic cell carcinoma. Although relatively rare in developed countries, hepatic cell carcinoma is one of the leading causes of death from cancer in Africa and Asia, and there is considerable evidence that HBV is a causative factor in such cancer. For these reasons the control of HBV is an important public health goal.

Research in the Hepatitis Viruses Section as well as in academic and industrial laboratories elsewhere over the past decade has led to the recent development and licensing of hepatitis B vaccines in the United States and France. Such vaccines are prepared from the plasma of individuals chronically infected with HBV. They have been shown to be both safe and effective in preventing type B hepatitis, but problems encountered in the collection of appropriate plasma, preparation of vaccine and safety testing of the final product will limit the availability of vaccine and will make it very expensive. For these reasons, work continues on optimum formulations of vaccine and dosage regimens and on the development of "second generation" and "third generation" vaccines. These newer vaccines will probably be produced in prokaryotic cells or yeast by recombinant DNA technology or they will consist of synthetic polypeptides.

Clinical testing and characterization of plasma-derived vaccines prepared by the NIH will soon be completed. An alum-adjuvanted preparation was found to be highly immunogenic and well tolerated in adult volunteers. Sixty-eight percent of vaccinees developed antibody within one month of vaccination and 95 percent seroconverted following completion of the six-month vaccination schedule. Several sublots have been prepared in an identical manner from the same master lot of vaccine; all have been similarly immunogenic in volunteers (Purcell, Ticehurst).

The rapid antibody response to the NIH vaccine suggested that it may be useful in preventing perinatal transmission of HBV from infected mothers to their offspring, an event that occurs frequently in Asia. In preparation for an efficacy trial of the vaccine in high-risk infants, immunogenicity tests were carried out by Dr. B. Hollinger (Baylor School of Medicine) with the NIH vaccine. The vaccine was administered to 65 children, 1-17 years old. The NIH vaccine was more immunogenic in young children than in adults: 53 percent of children 11-17 years old developed antibody within two weeks of vaccination, whereas 78 percent of children 1-2 years of age seroconverted by two weeks. By 8 weeks, 100 percent of children 1-10 years of age had developed antibody. Reactions to the vaccine were minimal and not significantly different from those seen in recipients of a placebo preparation. Thus, the NIH vaccine was highly immunogenic and nonreactogenic for children and infants.

Immunogenicity studies are being carried out with the NIH vaccine in the People's Republic of China and similar results have been obtained in adults and children. Immunogenicity trials in infants and newborns are in progress. An efficacy trial in newborn infants of mothers highly infectious for HBV will begin in the summer of 1982. The NIH vaccine and a Chinese vaccine will be compared with a placebo in a double-blind randomized study.

Synthetic polypeptides that define hepatitis B viral antigens are under study in collaboration with Dr. Richard Lerner (Scripps Institute). The amino acid sequence of such peptides was deduced from the nucleotide sequence of the HBV gene that codes for the surface antigen of the virus. Several polypeptides that represent different regions of the HBsAg gene had been shown previously to stimulate antibody reactive with the native surface antigen. A polypeptide derived from a variable region of the HBsAg gene was selected for additional studies in chimpanzees. This region is thought to define the subtype specificity of HBsAg. Three chimpanzees were vaccinated with a polypeptide (P-49) comprising amino acids 110-139 of the HBsAg gene product. The polypeptide was coupled to keyhole limpet hemocyanine carrier and adjuvanted with alum, pertussis organisms or Freund's incomplete adjuvant. Each of the chimpanzees developed antibody within two weeks of vaccination, and the response appeared to be directed against the subtype specificity (Y) of the antigen. However, the antibody response was relatively short-lived and attempts to "boost" the response with subsequent doses of the polypeptide were only partially successful.

The probable etiological association between hepatitis B virus (HBV) infection and subsequent development of hepatic cell carcinoma (HCC) suggests the possibility that "neoantigens" may be present in HBV-infected cells or in HCC cells that contain the HBV genome. Traditionally, these have been sought by employing as an antibody reagent the serum of a tumor-bearing animal. Man and certain higher primates are the only species known to be susceptible to HBV infection, and only man has developed documented HCC. Thus, patients with HCC may serve as a source of antibody for a hypothetical HBV neoantigen. Sera from patients with HCC from Japan and South Africa were tested for such an antibody by immunofluorescence using human HCC cell lines. Antibodies were detected in a small proportion of patients. These antibodies reacted with HCC cell lines containing integrated HBV genome but not HCC cell lines that lacked the HBV genome. In addition, the nuclear antigen was detected in one of seven HCC tumors from Taiwan (Johnson, Purcell). Antibody to the hepatitis B virus-associated nuclear antigen (HBNA) was detected in seven percent of patients with HBV-associated HCC, but not in patients with HBV-negative HCC (Wen Yu-Mei). In addition, low-titered antibody was found in two percent of patients with chronic HBV infection in the absence of HCC, but not in normal controls lacking serologic evidence of prior HBV exposure. These results are consistent with reports that HBV DNA is usually integrated into chromosomal DNA of HCC cells and that such integrated HBV DNA is also found in a portion of patients with long-term chronic HBV infection in the absence of detectable HCC (Wen Yu-Mei, Purcell).

Woodchuck Hepatitis Virus (WVH) - The similarities between HBV and WHV, coupled with the tendency of each virus to cause chronic hepatitis and hepatic cell carcinoma in their respective hosts, makes WHV and its host, the woodchuck, a particularly interesting model system. Sensitive radioimmunoassays for woodchuck hepatitis virus surface antigen (WHsAg) and its antibody (anti-WHs) were developed last year. These permitted an evaluation of a colony of wild-caught woodchucks for markers of infection. To further characterize WHV relationships, a colony of woodchucks has been established under contract with Cornell Veterinary School (Dr. B. Tennant, N.Y.S.C.V.M.). Activities for the first year of the contract included the establishment of the colony, the characterization of the woodchuck in captivity and the development of methods for the successful handling, breeding and manipulation of these animals. During the second year of the contract now in progress, an attempt to establish chronic WHV carriage by perinatal infection of offspring is in progress. In addition, a "vaccine" prepared from WHsAg in a manner similar to the hepatitis B vaccine, has been administered to selected newborn animals. Other animals have received a placebo. Certain of these animals were also inoculated with live WHV at birth and other offspring were naturally exposed to their WHV-positive mothers. The purpose of the study, which will last several years, is to determine the ability of vaccine to interrupt the maternal-fetal transmission of this hepadna virus in an attempt to predict the results of similar vaccine interruption trials with hepatitis B vaccine in man. The ultimate goal will be to prevent hepatic cell carcinoma by prevention of chronic virus infection of the newborn. Such results will not be obtained from vaccine trials in man for 20 to 40 years (the incubation period from infection to development of HCC). However, the incubation period to HCC in the woodchuck is only approximately 2 to 5 years. Thus, important prognostic information should be available from the woodchuck-WHV model system within the next few years. This information will be useful in planning for future use of hepatitis B vaccines in man (Purcell).

Delta Agent - In 1977, Mario Rizzetto discovered a new antigen in patients with chronic type B hepatitis. This antigen, detected by fluorescence, was found in hepatocyte nuclei of patients with chronic type B hepatitis. Its distinctness from hepatitis B core antigen was demonstrated. Extensive studies of delta antigen were carried out by Dr. Rizzetto when he was a Fogarty Visiting Scientist in LID. Most of these studies involved intensive collaboration with Dr. Gerin's laboratory of Georgetown University. Delta was found to be associated with a transmissible agent that required HBV synthesis for its own synthesis and that suppressed the synthesis of HBV markers. Thus, it had the characteristics of a defective interfering agent. Delta could be detected by immunofluorescence in the liver of chimpanzees experimentally infected with the agent, but could not be detected in the serum until centrifugal pellets of the serum samples were treated with detergent. Thus, delta appeared to be enclosed within a particle. When samples of chimpanzee plasma containing delta were subjected to centrifugal separation, delta copurified with 35-37nm particles that resembled large HBsAg particles. Furthermore, these particles were coaggregated with the 22nm HBsAg particles by anti-HBs. Thus, delta was an internal component of particles incapsidated by HBsAg. When such particles were extracted for nucleic acid, a low molecular weight (approximately 500,000 daltons) species was detected. This RNA also appeared to be enclosed, with delta, inside a coat of HBsAg. Delta was readily transmitted to chimpanzees either acutely or chronically infected with HBV, but could not be transmitted to HBV-immune chimpanzees. Light and electronmicrographic changes similar to those seen during acute non-A, non-B hepatitis infection have been detected in such chimpanzees, suggesting that delta may share some characteristics in common with other non-A, non-B agents (Purcell).

Recently, in a collaborative study with Dr. S. Hadler (CDC), Dr. Antonio Ponzetto (Turin, Italy), found serologic evidence for an etiological association between the delta agent and severe hepatitis with a high mortality in Yucpa Indians living in western Venezuela. Previous epidemiologic evaluation of the Venezuelan hepatitis had suggested an association with HBV infection, but a high prevalence of serologic markers of HBV infection in non-affected villagers made the association tenuous. However, when sera were tested for evidence of delta infection, virtually every patient who had experienced the severe form of hepatitis was found to have evidence of infection with the delta agent. In contrast, virtually none of the HBV-positive individuals in other villages had such evidence. Death from the Venezuelan hepatitis resulted from both fulminant hepatitis and a rapidly progressive form of subacute and chronic active hepatitis. It is not yet clear whether the Delta agent was introduced into this indigenous population recently or in the distant past. The delta agent is highly endemic in southern Italy and possibly other Mediterranean countries, and it is possible that the agent may have been introduced by individuals from such countries centuries ago. Indeed, there are rumors of outbreaks of similar severe hepatitis in Columbia. Extensive seroepidemiological studies will have to be carried out in various South American countries to determine the extent and medical importance of infection with the delta agent in this region. The emerging picture of the delta agent as a pathogen capable of producing severe hepatitis in carriers of HBV has prompted a search in the United States for similar cases. Evidence for such superinfection of HBV carriers, with resultant severe, life-threatening hepatitis, has been obtained in Dallas, Texas, Los Angeles, California, and Bethesda, Maryland. It is likely that, when serologic tests for delta infection are widely available, the agent will be found to be more widespread than previously thought.

Characterization of the agent and delta-associated hepatitis has continued in the chimpanzee model. Infectivity titrations of acute-phase chimpanzee plasma has revealed an infectivity titer of delta of at least 10^9 infectious units per milliliter and approximately 10^6 infectious doses of HBV per milliliter. Thus, we now have available an inoculum that contains at least 1000-fold higher titer of delta than HBV. This inoculum will permit us to determine unequivocally whether delta can infect susceptible hosts in the absence of HBV and whether other viruses (e.g., HAV, non-A, non-B agents) can serve as helpers for delta replication (Purcell).

That viruses other than HBV can serve as helpers for delta replication has been demonstrated by transmitting the delta agent from a HBV-carrier chimpanzee to a woodchuck chronically infected with the woodchuck hepatitis virus. A subsequent second passage of delta to a woodchuck chronically infected with WHV has also been achieved. The delta agent recovered from the woodchuck had the biophysical characteristics of the parent agent (Purcell).

In collaboration with Dr. J. Gerin (Georgetown University), attempts to clone the genome of the delta agent are in progress. Approximately 25 percent of the putative genome has been cloned; hybridization experiments between HBV and the delta agent, using cloned probes of the two genomes, have failed to reveal a relationship between HBV DNA and delta RNA.

Non-A, Non-B Hepatitis (NANBH) - Non-A, Non-B hepatitis continues to be an enigma for workers in this field. Extensive efforts to find a virus or an antigen related to this disease have been unsuccessful in our laboratory as well as many others around the world. We have continued to use immunologic methods to search for NANBH agents in collaboration with Drs. Harvey Alter and Hiro Shiraishi of the CC Blood Bank. However, extensive attempts using radioimmunoassays have been unsuccessful Feinstone, Purcell).

The animal models for NANBH, the chimpanzee and marmoset, offer systems in which the agent can be characterized. However, since a mild liver disease is the only measure of infectivity, the experiments must be performed with extreme care with well characterized infectious inocula and appropriate controls. Previously, the infectivity titer of two human plasmas infectious for chimpanzees was determined. One strain F) had a titer of less than 10^2 chimpanzee infectious doses (CID) per ml. The other (strain H) had at least 10^6 CID/ml. Other infectious inocula recently reported in the literature have a titer of 10^3 or less. The availability of a high-titered inoculum makes it possible for us to perform certain characterization experiments that would not be practical with low-titered material. Recently it was shown that the strain H NANBH agent was sensitive to the lipid solvent, chloroform. This suggests the NANB agent possesses a lipid containing coat. In the same experiment, it was shown that hepatitis B virus was chloroform-sensitive. A sham inactivated NANBH sample was later shown to be infectious in the chimpanzee that did not develop illness following inoculation with the chloroform-treated sample. Further characterization experiments, including sizing by filtration, are in progress. It is hoped that these experiments will lead to a rational approach to the detection of the agent (Feinstone, Purcell).

RESPIRATORY SYNCYTIAL (RS) VIRUS

Respiratory syncytial (RS) virus is a pleomorphic, enveloped, negative-stranded virus that is classified as a pneumovirus. It is the major respiratory tract viral pathogen of early life and hence is of considerable public health interest. Control of this virus poses several special problems because it has its greatest impact during the first six months of life, and it continues to reinfect individuals throughout their life. Immunity to severe disease is acquired through repeated infection but this immunity does not provide complete protection against reinfection.

Previous attempts to prevent disease with an inactivated virus vaccine failed, probably because: (a) formalin inactivation destroyed the F glycoprotein that plays an important role in immunity and (b) parenteral immunization did not induce effective local immunity in the respiratory tract. Failure of the inactivated vaccine stimulated interest in the development of a live attenuated RS virus mutant that could be used to induce infection immunity that includes local as well as systemic resistance and a cell-mediated response as well as an immunoglobulin response. This effort was also unsuccessful. Missense attenuated mutants were produced by chemical mutagenesis but these *ts* mutants proved to be unstable genetically as indicated by a loss of the *ts* phenotype during growth of virus *in vivo*.

These failures prompted a reevaluation of the RS virus genetics program. The decision was made to concentrate our efforts on molecular characterization of the viral genome and its products. Also, an intensive effort will be made to express certain viral DNAs in both procaryotic and eukaryotic cells with the intent of producing purified antigen that can be evaluated for its protective effect in vivo.

Cloning of RS Virus Genes - Respiratory syncytial virus grows poorly in vitro and remains predominantly cell associated. By manipulating the conditions of infection in cell culture we were able to detect viral specific polypeptides corresponding to nucleocapsid protein (44kd), matrix protein (28kd), and phosphoprotein (33kd) and two glycosylated surface proteins (84kd and 68kd). The latter glycoprotein may represent the fusion factor by analogy with a similar glycoprotein present in the paramyxoviruses. mRNA isolated from cells infected with RS virus was translated in vitro to yield polypeptides corresponding to the nucleocapsid protein, matrix protein and phosphoprotein as well as one other nonstructural viral polypeptide. The identity of three of these polypeptides was established by comparing them with authentic virus polypeptides by two dimensional peptide fingerprinting.

A cDNA library of mRNA from RS virus infected cells was constructed in E. coli using a plasmid vector and oligo dG/dC tailing. The second cDNA strand was transcribed by a method that avoided the use of S1 nuclease and self priming. From this cDNA library a series of recombinant plasmids was selected by a combination of methods. These included: (a) selection of plasmid DNAs reacting specifically with viral specific $[^{32}P]$ labeled mRNA, (b) hybrid selection of mRNAs by recombinant cDNAs and translation of these mRNAs and (c) hybrid arrested translation of viral mRNAs by the recombinant plasmids. These screening procedures allowed us to identify recombinant plasmids containing coding sequences for viral specific proteins. At least 4 of the RS viral genes appear to have been successfully cloned in this initial effort. These plasmids are currently being analyzed to determine their DNA sequence and to establish the reading frame of the various polypeptides (Venkatesan, Elango).

Although cloned DNA representing the viral surface glycoproteins has not been identified, it should be possible to produce the desired glycoprotein DNA clones by "walking across" the viral RNA genome (i.e., using cloned DNA as a primer to extend reverse transcription of DNA sequences into an adjacent region of the genome). Optimism for this approach is based upon the fact that the gene order for parainfluenza viruses appears to be 5' NP, P, Fo, M, HN 3'. If RS virus has the same gene order, clones coding for the NP, P, and M genes could be extended into the adjacent genes on the negative-strand template and subsequently amplified by cloning in E. coli. Sequencing of these clones before and after extension would allow us to define intergenic regions of the genome. Furthermore, the deduced amino acid sequence of the cloned glycoprotein genes could be compared to that of virion glycoprotein (Venkatesan, Elango)

Our interest in RS virus surface glycoproteins stems from the critical role they play in virus attachment, virus penetration, host range and tissue specificity. Equally important is the critical role these surface polypeptides play in the induction of immunity to infection and resistance to disease.

Immunity to RS Virus - A recent report from the Center for Disease Control stated that a recent isolate of RS virus (#9320) was distinct antigenically from established prototype strains, as judged by in-vitro cross-neutralization studies. For this reason, we undertook studies to determine the relatedness of the new isolate to five other strains of RS virus isolated over a twenty-five year period. Antiserum to each strain was prepared by intranasal inoculation of the virus into cotton rats, followed three weeks thereafter by exsanguination. In-vitro cross-neutralization studies, using all combinations of the six viruses and six antisera, are currently in progress. Preliminary assays have failed to show a significant difference among the strains tested thus far (Prince, Chanock).

Previously, we observed that active immunity to RS virus is of a different nature in the lungs than in the nose, pulmonary immunity being more durable. The nature of active immunity was then studied using the technique of parabiosis, in which surgically linked cotton rats develop cross-circulation of blood. Control animals, when linked to immune partners, were rendered immune to subsequent infection of their lungs but not their nose. Data from parabiosis clearly demonstrated a systemic component to pulmonary immunity that was not active in nasal tissues. Efforts are currently in progress to determine the blood-borne effector of pulmonary immunity. Preliminary results from serum-transfer experiments suggest that antibody, or some other humoral factor, plays a role in pulmonary immunity in parabiosis. Additional experiments in which immune lymphocytes were transferred failed to demonstrate a protective effect. Maternally-transmitted immunity to RS virus was also studied in cotton rats because a recent epidemiologic study of infants and children suggested that breast feeding conferred demonstrable resistance to serious RS virus respiratory tract disease during the first two years of life. Infant cotton rats born to and nursed on immune mothers exhibited partial immunity in the nose and near-complete immunity in the lungs. Foster-nursing experiments showed that there was a prenatal transfer of RS virus antibody, but this was not associated with resistance in the nose. Some resistance was conferred on the lungs but its effectiveness was slight. In contrast, postnatal immune transfer (through the colostrum and milk) conferred partial nasal immunity and near-complete pulmonary immunity. Thus, infants born to non-immune mothers, but nursed by immune mothers, exhibited the same degree of resistance as infants born to and nursed by immune mothers. It would appear that immunity transferred during the post natal period by colostrum and milk is considerably more effective than immunity transferred by the mother during pregnancy. Maternally transmitted immunity is of short duration, lasting one week in the nose, and four weeks in the lungs. The effectors of this immunity have not been identified. However, the lack of correlation between antibody level in infants and resistance to infection suggests a cellular component (Prince, Chanock).

INFLUENZA A VIRUS

Genetic Approaches to Construction of Attenuated Viruses - Viruses bearing specific, identifiable, attenuating mutations represent the live vaccine strains of the future because the genetic basis for attenuation is known and can be monitored directly during all phases of vaccine development, manufacture, and utilization in humans. The greatest success in producing and characterizing such attenuating mutations has been achieved with influenza A virus, whose segmented genome facilitates the segregation and characterization of individual genes. Previously we used chemical mutagenesis to produce temperature-sensitive (ts) mutations on genes that code for nonsurface viral proteins so that the mutant genes could be transferred into reassortant viruses bearing the surface antigens of new epidemic or pandemic strains. Although the ts genes from one of our donor viruses, ts-1A2, conferred a satisfactory level of attenuation upon a series of reassortant viruses, it became clear that genetic instability represented an insoluble problem. During infection of a susceptible child, one of the *ts*-1A2 reassortants underwent a complex set of genetic alterations, including suppression, that led to loss of the *ts* phenotype and restoration of virulence, even though the *ts* reassortant was greatly restricted in its ability to replicate *in vivo*.

The genetic stability of a new "*ts*-1[E] like" virus (*ts* P3 and *ts* NP genes) was compared with that of the *ts*-1A2 donor virus *in vitro* in a temperature stress test and *in vivo* in cyclophosphamide treated hamsters. The new reassortant was more stable than the *ts*-1A2 virus *in vitro* and *in vivo*. However, it still underwent progressive loss of its temperature sensitivity in both test systems to an extent that would preclude further evaluation of this virus as a donor of its attenuating genes to new epidemic human viruses. Thus, all work with *ts* mutants of influenza A virus produced by chemical mutagenesis has been discontinued (Murphy).

Reassortant Viruses Derived from a Virulent Human Influenza A Virus and an Attenuated Avian Influenza A Virus - Clearly, there is a need for stable attenuated viruses that are unable to escape their attenuated phenotype. Many of the influenza A virus genes that have evolved over a long period of time in birds differ significantly from corresponding genes of human influenza A virus. Because of these marked differences, we would anticipate that some avian influenza viruses would replicate inefficiently in human cells and thereby be attenuated. It would also be anticipated that such attenuated avian viruses would retain their attenuated characteristics after limited replication in man.

This concept was evaluated initially in squirrel monkeys because these primates develop illness after experimental infection with human influenza A viruses. In a previous study we identified an avian virus that was infectious but avirulent for squirrel monkeys. During the past year we sought to attenuate a virulent human influenza A virus for monkeys by substituting the 6 non-surface antigen genes of the attenuated avian influenza virus for the corresponding genes of the human virus. This method of generating attenuated viruses by the production of a hybrid virus containing the hemagglutinin (HA) and neuraminidase (NA) genes from a human influenza A virus and the other 6 genes from a non-human influenza virus represents a new approach to the production of viral vaccines for man (Murphy, Chanock).

The avian influenza A virus [A/Mallard/N.Y./6750/78 (H2N2) virus] selected as the donor of attenuating genes was approximately 1000-fold more restricted in its replication in primates than virulent human influenza A viruses. The avian A/Mal/N.Y./78 virus was mated with the human A/Udorn/72 (H3N2) wild type virus and reassortant viruses containing genes coding for the 2 surface antigens of the human virus and the internal genes of the avian influenza A virus were sought. Progeny viruses were selected by passage and plaque isolation in primary chick kidney culture at 42°C (a temperature restrictive for human influenza A virus) in the presence of antiserum to the H2 hemagglutinin (HA) and the N2 neuraminadase (NA). The progeny derived from six plaques were genotyped and five clones possessed the desired gene constellation, i.e., the HA and NA genes came from the human influenza parent and the six "internal" genes were derived from the avian influenza parent. These results indicated that one or more of the six transferred avian genes specify the capacity for viral replication at 42° and that restriction of the human influenza A virus in avian tissue can not be attributed to its HA and NA genes. These two observations provide the basis for rapid selection of a desired reassortant virus that possesses the two human influenza surface genes and the six avian influenza internal genes. Importantly, reassortant virus bearing the avian virus internal genes grows to a titer between 10^8 and 10^9 TCID$_{50}$/ml in the allantoic cavity of eggs.

One of the reassortant viruses with 6 internal avian genes was evaluated in squirrel monkeys. The reassortant virus was found to be as restricted in its level of replication in the upper and lower respiratory tract as its avian influenza virus parent. In addition, the reassortant was as attenuated as its avian influenza parent. Nonetheless, infection with the reassortant induced significant resistance to challenge with virulent human influenza virus. It appears that the non-surface antigen genes of the avian parental virus are primary determinants of restriction of replication of the reassortant virus in monkeys. Our findings form the basis for a new approach to the development of a live virus vaccine in which the genes coding for the non-surface antigens of an attenuated avian virus are substituted for the corresponding genes of a virulent human influenza virus. In this manner, attenuation is achieved by the inefficient functioning of the avian influenza genes in primate cells, while antigenic specificity of the human influenza virus is provided by the neuraminidase and hemagglutinin genes derived from the human virus. This approach could lead to the development of a live influenza A virus vaccine that is attenuated for man if the avian influenza genes are similarly restricted in human cells (Murphy, Chanock).

Evaluation of Cold Adapted (ca) Influenza A Virus Reassortants in Volunteers - Another technique now being actively pursued for rapid attenuation of new influenza A viruses involves the use of a donor virus that grows well at a suboptimal temperature that does not support efficient replication of wild-type virus. The A/Ann Arbor/6/60 (H2N2) donor strain was adapted by Maassab of the University of Michigan to grow well at 25° by serial passage at successively lower temperatures (33°C-25°C) in primary chick kidney cell culture. This virus was also shown to be *ts* with a shutoff temperature for plaque formation of 38°C. Genes from this cold-adapted (*ca*) donor strain were transferred to a succession of new antigenic variants of influenza A virus, and reassortant viruses bearing the *ca* and *ts* properties of the attenuated donor virus were isolated. Ideally, reassortant viruses produced by mating the A/Ann Arbor/6/60 *ca* donor and a new epidemic influenza A virus should receive all their nonsurface viral protein genes from the *ca* donor and their surface glycoprotein genes from the new variant. Maassab and his associates have produced *ca* reassortants with this gene constellation by mating the A/Ann Arbor/6/60 *ca* donor and wild-type virus at 25°C, a temperature restrictive for replication of wild-type virus.

During the past year two *ca* reassortants, one bearing contemporary H1N1 antigens and the other contemporary H3N2 antigens, were subjected to extensive evaluation in seronegative volunteers. These studies were performed in collaboration with Drs. Clements (University of Maryland), Betts (University of Rochester) and Dolin (University of Vermont). Both reassortants were satisfactorily attenuated and stable genetically. Approximately $10^{6.8}$ to $10^{7.5}$ TCID$_{50}$ of virus were required to infect 80 percent of seronegative volunteers. The human infectious dose$_{50}$ was approximately $10^{5.5}$ to $10^{6.0}$ TCID$_{50}$. These very encouraging findings from the collaborative study suggest that *ca* reassortants of influenza A virus bearing the 6 internal genes of the *ca* donor virus show considerable promise for use in immunoprophylaxis of influenza in man (Murphy).

Probing the Internal Proteins of Influenza A Virus with Monoclonal Antibodies - Little is known about the antigenic and functional properties of the internal proteins of influenza viruses, namely the nucleoprotein, matrix, and three polymerase proteins. Since monoclonal antibodies prepared to the nucleoprotein have enabled us to study in detail antigenic variation of this protein in influenza viruses and to define topographical domains of the nucleoprotein that are involved in RNA transcription, we have chosen this approach to also study the other internal proteins of influenza viruses, namely the matrix and polymerase proteins.

Mice immunized with sucrose gradient fractions of disrupted virus enriched in polymerase proteins developed serum antibodies that immunoprecipitated the polymerase proteins and were capable of inhibiting viral RNA transcriptase activity *in vitro*. The spleens of these mice were used to prepare approximately 400 monoclonal cell lines. The antibodies produced by these cell lines were screened in routine serologic assays (hemagglutination-inhibition, enzyme-linked immunosorbent assays, fluorescent antibody tests, and radio-immunoprecipitation) as well as *in vitro* RNA transcriptase inhibition assays. Five monoclonal antibodies were found to be active in transcriptase-inhibition tests, but were not positive by the routine serologic analyses described above. These antibodies, which may prove to be directed to one of the influenza virus polymerase proteins, are currently being studied. Since the specificities of these antibodies were not revealed using radio-immunoprecipitation assays, we used Western blot analyses. Using this method we determined that these antibodies bind to a protein that has an approximate molecular weight of 90.000 daltons and that co-migrates with influenza polymerase proteins. These antibodies appear to be broadly cross-reactive; they also inhibit RNA transcription of influenza B viruses. However, they do appear to be influenza-specific since they do not inhibit the transcriptase of measles virus. Additional experiments are in progress to determine unequivocally the specificities and functional properties of these antibodies. Parallel studies on the matrix protein are also in progress in collaboration with Dr. Jonathon Yewdell of the Wistar Institute. Six monoclonal antibodies are being used to study antigenic variation in the matrix protein of human and animal influenza viruses. Preliminary results indicate that little antigenic change occurs in the matrix proteins of influenza A viruses, and that the matrix protein is not structurally important for the maintainence of ribonucleoprotein-complex stability during RNA transcription (van Wyke).

MOLECULAR BIOLOGY OF INFLUENZA VIRUS

The RNA genome of influenza virus contains eight single stranded RNA segments that are of negative polarity. During infection of a permissive cell, these virion RNA segments are transcribed and polyadenylated, generating corresponding positively-stranded mRNA molecules. Full-length nonpolyadenylated viral RNA species are also transcribed, and these function as templates for virion RNA synthesis. Each of the virion RNA segments codes for a single protein except for segments 7 and 8 that code for two (or more) proteins. Segment 7 encodes M_1 and M_2, while segment 8 encodes NS_1 and NS_2. Immunological response to the two surface glycoproteins (hemagglutinin (HA) and neuraminidase (NA)) is a primary factor in determining the host's resistance or susceptibility to the virus. Alteration in the HA, and to a lesser extent the NA, is a prominent feature of new antigenic variants of influenza viruses that are responsible for pandemics or epidemics. There is a need to broaden our knowledge of the genetics of the influenza viruses in order to design ways to control these viral pathogens. One approach is to employ recombinant DNA techniques that have had considerable success in elucidating the molecular biology of many DNA viruses as well as RNA viruses.

Recently, cloned, full-length DNA derived from the single stranded RNA genome of poliovirus was shown by Racaniello and Baltimore to be infectious. This means that mutations introduced at strategic locations in the poliovirus genome can now be studied for their effect on viral structure, function and virulence. Similarly, our goal with influenza virus is to devise procedures that would permit conversion of cloned influenza DNA back to influenza virus negative strand RNA and eventually transfer such RNA into an influenza virus. In this manner, stable site-specific mutations, such as deletions, induced in the cloned DNA, might be transferred back to the influenza virus. Thus, it may be possible to develop stable mutants for experimental study and for use in immunoprophylaxis.

Cloning DNA Sequences from Influenza Virus Genes - Techniques were developed to clone DNA containing complete sequences of an influenza A virus of the H3N2 subtype (Udorn/1972). Initially, cDNA strands were synthesized separately by reverse-transcription of genomic RNA segments using a specific dodecadeoxyribonucleotide complementary to the 3'-terminus as a primer and by reverse-transcription of cytoplasmic virus mRNA using an oligo (dT) primer. DNA duplexes were formed from corresponding cDNA copies and inserted into the Pst 1 site of plasmid pBR322 for transformation of E. coli. These cloned DNA segments also contained dG/dC linkers at their termini, since these linkers were added during construction of the recombinant plasmids. Full-length DNA clones derived from four of the eight gene segments of influenza virus (A/Udorn/72 H3N2) were obtained. These four segments code for the hemagglutinin (HA), neuraminidase (NA), matrix protein (M), and nonstructural proteins (NS). Recently, a second cloning method was employed to obtain two additional full-length DNA clones, coding respectively for the nucleoprotein (NP) and the polymerase protein that binds to the mRNA cap structure (P3). The latter approach involved the use of two separate oliogonucleotide primers, a dodecadeoxyribonucleotide that primes cDNA synthesis from vRNA (as described above) and another dodecadeoxyribonucleotide that represents the conserved sequences at the 5' terminus of vRNA. The latter primer was used to initiate second-strand DNA synthesis. The resulting full-length duplex DNA was used for cloning in pBR322 using Bam HI linkers. Full-length DNA segments coding for NS, M and NA, respectively, were also produced by this technique. The double primer method yielded DNA segments that did not contain G/C linker sequences (Lai, Lin, Markoff, Sveda).

Complete Nucleotide Sequence of Neuraminidase The NA gene was cloned at the Pst 1 site of the plasmid vector pBR322 after G-C tailing of plasmid and influenza DNA. A restriction endonuclease map was determined, and these data provided the basis for the complete sequencing of the NA DNA by the chemical method of Maxam and Gilbert. The neuraminidase DNA had a total length of 1,466 bases, excluding G-C linker residues. The cloned segment retained the common sequences known to be present at the 3' and 5' termini of virion RNA. Also present in one of the NA clones analyzed was a nonviral oligonucleotide 9 bases long at the 5' (+) end of the DNA that represents cellular sequences acquired during influenza mRNA synthesis. The sense strand of the DNA contained a single open reading frame coding for 469 amino acids. A possible polyadenylation site was noted downstream from the termination codon. Mammalian gene splice sequences or an alternate open reading frame were not present. The deduced amino acid sequence displayed the features of other neuraminidase genes that had been partially or completely sequenced, including the conservation of a sequence of 12 amino acids at the NH_2-terminus and the presence of a hydrophobic region from amino acid positions 7 to 34. The data were consistent with the hypothesis that this hydrophobic region of the surface glycoprotein represents the site for membrane insertion. Our findings indicate that the N2 gene sequence differs from that of an N1 gene (previously sequenced by others) by numerous point mutations, as well as by insertion/deletion. This means that antigenic "shift" in the human neuraminidase antigenic subtype in 1957 most likely occurred by replacement of the human N1 gene with an allele from the animal and avian reservoir of influenza A viruses; this occurred presumably through gene reassortment Markoff).

Construction of SV40-Influenza Viral DNA Recombinants for Expression of Influenza Proteins - SV40 DNA has been widely used as a vector for introduction of cloned DNA into mammalian cells. The SV40-African green monkey kidney (AGMK) system is particularly attractive for the recombinant DNA manipulation of influenza virus. First, recombinants containing SV40 and an influenza DNA insert (positioned in the late region of SV40) synthesize stable RNA transcripts that contain influenza viral sequences under the transcriptional control of SV40. Depending on the orientation of the influenza DNA insert with respect to the direction of SV40 transcription, either the (+) or the (-) strand of influenza viral RNA is produced in cells infected with the recombinant. Second, African green monkey kidney cells are permissive for replication of both SV40 and influenza virus. Thus, coinfection of AGMK cells with an SV40-influenza DNA recombinant and an influenza virus could conceivably lead to allele replacement, especially if the coinfecting influenza virus contains a selective marker in the allelic gene segment.

To achieve this goal, cloned full-length HA DNA was initially inserted into the late region between the Hae II site and the Bam HI site of SV40. Recombinant molecules were propagated in the presence of a helper SV40 early ts mutant (ts A28). Analysis of SV40-HA recombinants showed that HA inserts in both orientations were obtained.

More recently, an expression vector containing a single Bam HI site for insertion of a cloned gene segment in the late region between the Hpa II and the Bam H1 site of SV40 was constructed by Dr. P. Gruss and made available to us. This vector was employed to produce recombinants of other influenza viral gene segments that contain Bam HI linkers at their termini. So far, recombinants successfully propagated in the presence of a helper SV40 ts mutant included SV40-HA, SV40-NA, SV40-M, SV40-NP, and SV40-P3. With the HA and NA genes we produced at least one recombinant with a (-) orientation insert and at least one recombinant with a (+) orientation insert. We characterized in some detail those recombinants that synthesize the (+) strand influenza viral RNA in a series of investigations concerning transcription, and expression of influenza viral genes (Lai, Markoff, Lin, Sveda).

Expression of Influenza Proteins from Cloned DNA and Effect of Selected Deletions on Structure and Function of These Proteins: Hemagglutinin - Influenza virus acquires its hemagglutinin (HA) during the final stage of viral maturation at the cell surface. The HA, encoded by viral gene segment 4 is a trimeric molecule (HA_0 subunit, M_r 70,000-75,000) that projects from the surface of the virion. This surface projection causes erythrocytes to agglutinate and is responsible for the binding of virus to cell surface receptors during the initial stage of infection. Immunological response to the HA antigen is a primary factor in determining the host's resistance or susceptibility to the virus. Alteration in the HA (and to a lesser extent, the neuraminadase) is a prominent feature of new antigenic variants of influenza A virus responsible for pandemics or epidemics. The HA protein is synthesized in the cytoplasm of the infected cell and is then transported to the cellular membrane where it becomes anchored. Like many other membrane proteins, it undergoes post-translational processing that includes cleavage of a prepeptide and glycosylation of the molecule. We have recently obtained functional expression of the influenza virus hemagglutinin in mammalian cells from cloned full-length DNA inserted into an SV40 vector in the (+) sense orientation. Deletion mutations were then generated to determine whether the hydrophobic COOH-terminus of the influenza hemagglutinin is required for anchorage in the cell membrane.

Sequence analysis of the cloned DNA showed that the H3 subtype hemagglutinin, like the viral antigens of other subtypes, contained a hydrophobic region of 24 amino acids near the COOH-terminus of the HA molecule. In order to examine the functional role that the hydrophobic terminal sequences play in insertion of the viral protein into the lipid bilayer of the cellular membrane and, likewise, the virion surface, we constructed mutants of SV40-HA containing a deletion at or near the unique Bam HI site in the HA-DNA upstream from the region coding for the hydrophobic sequence. Bam HI cleaved linear SV40-HA DNA derived from form I SV40-HA DNA was treated with nuclease S1 to remove the cohesive ends. Primate cells were then infected with the treated DNA in the presence of complementing SV40 ts A mutant and a series of virus mutants containing deleted HA DNA were generated. The hemagglutinin mutant dl-2 (SV40-HA) sustained a deletion of 536 base pairs inclusive of the Bam HI site and all the coding sequences of the COOH-terminus. Mutant dl-9 (SV40-HA) lacked 489 nucleotides and contained an 11 base-pair insertion before the linker region. The third mutant, dl-8 SV40-HA), contained an insertion of approximately 300 base pairs beginning at nucleotide position 1610 (amino acid 511). The insertion sequence at the deletion junction codes for an additional 9 amino acids at the COOH-terminus of the HA polypeptide that is 520 amino acids in length. The dl-8 polypeptide therefore lacks the last 40 amino acids of the wild-type HA including the entire hydrophobic region. Each of the mutants produced a shortened HA polypeptide. In contrast to the full-length HA protein produced by SV40-HA, the shortened HA produced by the mutants could not be detected by fluorescent antibody staining of unfixed infected cells. Thus, the mutant HA molecules synthesized in the cytoplasm failed to accumulate at the cell surface, indicating that the HA was not anchored in the host cell membrane. Much of the shortened hemagglutinin polypeptide produced by the mutants, was secreted by the infected cell, whereas the wild type HA was completely cell-associated.

Removal of the hydrophobic carboxy-terminal sequences also directly or indirectly altered the glycosylation pattern of the mutant HA polypeptides. Thus, the carboxy-terminal sequences of HA play a role in glycosylation as well as membrane anchorage.

Finally, a fourth mutant, dl-12, sustained a frame shift at the beginning of the COOH-terminus resulting in the introduction of 6 charged amino acids into this domain that is normally hydrophobic. dl-12 HA was not secreted nor was it found on the host cells' outer membrane but it exhibited hemagglutinating activity. Unexpectedly, we found that the dl-12 HA localized at the inner aspect of the host cell membrane (Sveda, Lai).

Neuraminidase - Recombinant virus containing complete NA DNA in the (+) sense orientation with respect to SV40 late region promoters was used to study expression. Protein extracts from S^{35}-methionine labeled infected AGMK cells contained an immunoprecipitable polypeptide that co- migrated on SDS-polyacrylamide gels with authentic influenza NA. Fluorescent antibody studies involving live ("unfixed") infected cells indicated that the NA polypeptide was present on the host cell membrane. However, evidence that the NA polypeptide was functional was not obtained. Deletions were made in either the amino or carboxy termini of the NA gene to study their effects on glycosylation, transport, and membrane insertion. A frame-shift deletion mutant was constructed that contained a termination codon at map position 0.68. This mutant coded for a truncated polypeptide that lacked the 30 percent carboxy end of the NA DNA molecule. This truncated NA molecule was expressed on the membrane of the infected cell. Since deletion in the membrane anchorage region of the hemagglutinin gene usually results in secretion of mutant polypeptide (see Sveda *et al*), these data suggest that the amino acid sequences at the carboxy terminus of the polypeptide do not play a role in membrane insertion (Markoff, Sveda, Lai).

Nucleoprotein (NP)- Nucleoprotein interacts with viral genomic RNA to form helical ribonucleoprotein complexes. These complexes in association with the three viral polymerase proteins are transcriptionally active generating viral mRNA species. Recent evidence indicates that the nucleus is the site of influenza mRNA transcription and that NP is primarily present in the nucleus of infected cells.

During infection of AGMK cells, three independent SV40-NP recombinants produced a 56K dalton protein equivalent in size to the NP produced during influenza virus infection. Although there is no direct functional assay currently available for influenza virus NP, earlier studies indicated that NP is found inside the nuclei of infected cells. Similar to NP produced during infection by influenza virus, NP produced by SV40-NP recombinant localized primarily in the nucleus. Since other influenza viral polypeptides or vRNA segments were not present in SV40-NP infected cells, the specific influx of NP into the nucleus appears to be an intrinsic property of this polypeptide. Monoclonal antibodies specific to NP affect mRNA transcription *in vitro* and at least three non-overlapping antigenic determinants on the viral polypeptide have been demonstrated. In order to map the positions of these antigenic epitopes on the linear NP molecules, deletions were introduced into the NP gene so that shortened NP polypeptides were produced from the corresponding SV40-NP recombinants. Analysis by immunoprecipitation showed that the N-terminal half of NP appeared to react with monoclonal antibodies representing the three different classes, whereas the carboxyl half of NP did not react with the monoclonal antibodies. Of interest, cells infected with a SV40-NP deletion mutant (that coded for a shortened protein of 19K daltons at the N terminus of NP) expressed a small NP on the plasma membrane. In contrast, full-length NP was expressed in the nucleus of cells infected with SV40-NP that contained the full sequences for the NP protein. These findings may be relevant to the observation that NP can be detected by immunofluorescence, agglutination and microcytotoxicity assays on the surface of cells infected with influenza virus (Lin, Lai).

Transcription Control of Influenza A Virus: Synthesis of Spliced and Unspliced mRNAs - Splicing of influenza mRNA was first recognized two years ago during a collaborative study with Dr. Lamb (Rockefeller University). RNA segment 8 codes for two distinct proteins, NS_1 and NS_2, that are translated from separate mRNA species and RNA segment 7 codes for at least two distinct proteins, M_1 and M_2, that are also translated from separate mRNA species. Mapping and sequencing studies have shown that whereas the NS_1 and M_1 mRNA species are colinear transcripts of their respective RNA segments, the NS_2 and M_2 mRNAs contain an interrupted region. In addition to the M_2 mRNA, another interrupted mRNA (M_3 mRNA) derived from segment 7 has been identified. The coding potential of the M_3 mRNA is likely to be for only 9 amino acids, which would be identical to the C-terminal region of the matrix protein (M_1). The nucleotide sequences at the 5'-donor and 3'-acceptor sites of the interrupted mRNAs follow the "consensus sequence" pattern for spliced eukaryotic mRNAs and therefore it seems likely that these mRNAs are derived by splicing of their colinear transcripts NS_1 and M_1 mRNAs. Of interest, influenza virus does not have a known DNA intermediate involved in its transcription or replication that could serve as a template for spliced mRNAs. In this situation, splicing must occur during transcription from an RNA template. To demonstrate that the interrupted mRNAs derived from influenza virus RNA segment 7 are formed by RNA splicing, cloned DNA of RNA segment 7 (M DNA) was inserted into the late region of an SV40 vector, so that transcription of the M DNA was placed under the control of the SV40 promoter. Analysis of the mRNAs showed that both uninterrupted and interrupted influenza virus mRNAs containing both SV40 5' and 3'-terminal sequences were synthesized. Splicing of the recombinant derived influenza virus mRNA occurred with a donor site at M DNA nucleotide 11 and an acceptor site at nucleotide 740, and these are the same nucleotides used to form an interrupted mRNA (M_3 mRNA) during *in vivo* influenza virus transcription. This indicates that these sites in the influenza virus colinear transcript M_1 mRNA are substrates for splicing. Two interrupted mRNAs derived from influenza virus RNA segment 7 (M_2 mRNA and M_3 mRNA), using alternative donor sites but the same acceptor site, have been identified in influenza virus infected cells. That only the M_3 mRNA donor site was utilized in mRNA produced from the SV40-M vector may reflect constraints imposed by the SV40 5' or 3' nucleotide sequences. Influenza virus mRNAs are incomplete transcripts of the vRNA strand and polyadenylation occurs when transcription reaches the nucleotide sequence UUUUUU on the virion strand. As transcription of influenza virus RNA is mediated by the virion polymerase, it was of interest to determine whether the host cell polyadenylation mechanism would recognize this signal or whether polyadenylation would result from the SV40 signal, AAUAAA, and occur at the site used for SV40 virus late transcripts. The results of nuclease S1 mapping experiments with the SV40-M mRNAs are consistent with the interpretation that polyadenylation occurs at the normal SV40 site.

Attempts at Allele Replacement Using Cloned Influenza DNA - The SV40-HA (coding for the H3 subtype) and the SV40-NA (coding for the N2 subtype) recombinants used in the initial allele replacement studies produced a functional viral polypeptide, indicating that (+) strand RNA was produced in infected AGMK cells. To facilitate the detection of gene rescue from cloned influenza DNA, we used specific antiserum to suppress virus particles bearing the corresponding surface antigen coded by the rescuing virus. Primate cells were coinfected with SV40-HA or SV40-NA and influenza A virus of the H1N1 subtype (strain WSN). Infected cell lysates were then prepared and subsequently analysed for viral progeny that might contain the gene segment derived from the DNA recombinant. These experiments were carried out separately with either the SV40-HA recombinant or the SV40-NA recombinant. In each instance, antibody was employed to inhibit virus bearing the H1 antigen or N1 antigen of the coinfecting virus (depending on the cloned DNA under study) and thus favor the emergence of virus with the desired rescued allele. We were unable to detect reassortant virus in which H1 or N1 was replaced by H3 or N2 coded for by the SV40-HA or SV40-NA recombinant (Sveda, Markoff, Lai).

Although a full-length influenza viral sequence is present in our recombinant molecules of SV40-HA and SV40-NA, infection of AGMK cells with these recombinants also results in the synthesis of non-influenza RNA sequences that flank the influenza RNA transcripts see Transcriptional Control of Influenza A Virus). These flanking SV40 sequences are involved in initiation of RNA transcription (5') and polyadenylation of RNA transcripts (3'). These additional 5' and 3' SV40 sequences may make it difficult for allele replacement to take place in the system we have employed.

Currently, we are investigating the feasibility of influenza virus gene rescue using recombinants with influenza DNA in the (-) orientation. Recombinants that produce the (-) strand of influenza viral RNA are now being characterized for completeness of their influenza viral DNA inserts. Those recombinants that contain complete inserts will be utilized for the type of allele replacement experiments described earlier. Another approach involves cells persistently infected with cloned DNA and continuously expressing influenza viral RNA sequences. These persistently infected cells will be coinfected with another influenza A virus. This situation might allow the rare event of allele replacement to take place. Such a persistently infected cell system would circumvent the cytopathic effect of SV40 that eventually kills infected cells.

VIRAL GASTROENTERITIS

Norwalk and Related Viruses - Over the last 10 years the Epidemiology Section, LID, has been involved in the study of viral agents associated with epidemic diarrhea. Volunteer studies led to the characterization of a number of infectious inocula derived from several well studied outbreaks of nonbacterial gastroenteritis. The novel application of immune electron microscopy (IEM) to volunteer fecal specimens led in several instances, to the detection of the causative gastroenteritis agent. Norwalk virus, Hawaii agent, Marin County agent, and Snow Mountain agent are all serologically distinct 27 nm viruses that have been associated with epidemic diarrhea using the technique of IEM. The Norwalk particle has been investigated most thoroughly and is currently the prototype of the "small particle" diarrhea agents (Kapikian, Greenberg, Wyatt, Chanock).

None of the 27 nm gastroenteritis viruses has been cultivated in tissue culture. This means that the only virus available for study is that shed by infected individuals. Investigation of these viruses has been severely hampered by the small amount of virus shed during infection. This has prevented us from establishing the taxonomic status of these viruses. Nonetheless, limited biochemical analysis of Norwalk virus using ultracentrifugation and immunologic purification techniques indicates that the proteins of Norwalk virus most closely resembles those of the caliciviruses (Greenberg).

Meanwhile, studies of the other serologically distinct 27 nm particles have lagged behind. Recently, volunteer studies with the Marin County agent, a serologically distinct virus, yielded an unexpected result. Volunteers were administered a fecal filtrate derived from a patient who developed gastroenteritis during a nursing home outbreak in Marin County, California. One volunteer developed typical viral gastroenteritis (nausea, vomiting, malaise, and diarrhea) except that illness occurred late (6 days) after oral administration of the inoculum. This volunteer shed a large amount of virus, far in excess of that observed with any of the other 27 nm enteric viruses. IEM studies utilizing the volunteer's virus established that it was indeed the Marin County agent. Because of the large amount of virus in the volunteer's stools, it has been possible to partially purify it and establish a buoyant density (1.39 gm/cm^3). In addition guinea pigs immunized with the partially purified virus have made antibody that will be useful in developing 3rd generation solid phase immunoassays. Finally, it should be possible to characterize the virus chemically and physically and determine whether it contains an RNA or DNA genome (Greenberg, Kapikian).

ROTAVIRUSES

Because of their importance in severe and often fatal diarrheal illness, special emphasis has been given to characterization of these viruses at all levels. These ongoing efforts range from studies of the rotavirus genome and its protein products to epidemiologic investigations and clinical evaluation of a candidate rotavirus vaccine strain in adult volunteers.

Development of New Methods and Reagents for Studying Rotaviruses - A technique for RNA-RNA hybridization was developed to facilitate the comparison and characterization of each of the 11 rotavirus genes present in virus strains active at different times and in different places and derived from patients with disease of varying severity. For this purpose, highly labeled single stranded (ss) RNA transcripts were prepared from viral cores. (ss) RNA probes were then hybridized to denatured viral RNAs from strains under study, and the resulting double-stranded (ds) RNA hybrids were subjected to gel electrophoresis (PAGE). Exposure of the hybrids to S1 nuclease prior to PAGE increased the specificity of the test, since S1 digested non-homologous regions of the hybridized ds RNAs. This technique was also successfully applied to the assignment of parental origin of genes present in reassortant viruses (Flores).

Monoclonal antibodies directed against 5 of the rotavirus proteins were developed for use in clarifying rotavirus protein structure and function (Greenberg).

Phenotypic Analysis of Rotavirus Reassortants - Rotaviruses, as members of the reoviridae, have a segmented double-stranded RNA genome. Like reoviruses and myxoviruses, they undergo gene reassortment at high frequency during coinfection. Advantage was taken of this property to produce and isolate rotavirus reassortants to study the gene-product relationship of several important viral functions. In addition, it is possible that certain selected viral reassortants might be attenuated for man and thus represent vaccine candidates.

One (or both) of the parental viruses was a temperature-sensitive (ts) mutant. Selection for reassortant viruses produced during coinfection could thus be exerted by growing the yield from coinfected cultures at a temperature restrictive for the parental ts mutant(s). Similarly, virus with surface antigens of one of the parental viruses could be selected against by exposing the growth yield of coinfected cultures to specific antiserum directed against that parental virus. With these selection procedures, reassortants were produced by mating a bovine rotavirus with a rhesus monkey rotavirus or a human rotavirus. The reassortants were examined for phenotype by neutralization in tissue culture (neutralization specificity or serotype), subgroup antigen specificity, hemagglutination, and enhancement of infectivity by exposure to protease protease-enhanced infectivity). Genotype (i.e., parental origin of each of the 11 rotavirus genes) was determined by PAGE or RNA-RNA hybridization followed by PAGE. The following correlations were established: (1) serotype is coded by the 8th or 9th RNA gene, depending on the virus; (2) subgroup antigen is coded by the 6th RNA gene; (3) restriction of growth of human rotavirus in tissue culture, protease-enhanced infectivity and hemagglutination are all functions of a protein coded for by the 4th RNA segment. Independent segregation of serotype and hemagglutinin came as a surprise because in all other virus groups studied previously these two properties have been functions of the same protein (Kalica, Greenberg).

Localization of these proteins on well characterized reassortants (i.e., reassortants that had been completely genotyped) was achieved through the use of monoclonal antibodies to immunoprecipitate viral proteins that were then analyzed by PAGE. In each instance the corresponding genes of the parental viruses could be distinguished. Similarly, the parental viruses used to produce the reassortants differed as regards serotype, subgroup specificity, protease-enhanced infectivity and/or hemagglutination. In this manner the relevant gene and its product would be associated through the study of such well characterized reassortants. The 4th gene product was identified as the major outer capsid (82K) protein. The 6th gene product was determined to be the major inner capsid (42K) protein, while the 8th or 9th gene product was established to be a major outer capsid protein considerably smaller (37K) than the 4th gene product (Greenberg, Kalica).

Genetic Diversity of Rotaviruses - Genetic diversity was studied by comparing human and animal rotaviruses by RNA-RNA hybridization combined with PAGE. In this manner diversity of individual genes could be evaluated. Rotaviruses recovered from the same animal species exhibited a high degree of genetic homology, whereas viruses derived from different animal species differed significantly. For example, calf rotaviruses constituted a relatively homogenous group with a high degree of homology (80-100%). In contrast, calf rotaviruses differed significantly from rhesus monkey, porcine and human rotaviruses; in these instances heterospecies homology was partial, varying from 25 to 50%. The 2 rhesus monkey rotaviruses tested were completely homologous and differed significantly from the other animal rotaviruses as well as a variety of human rotaviruses. Finally, a murine rotavirus (EDIM) was quite distinct from all other rotaviruses tested (Flores).

An interesting pattern emerged among the human rotaviruses studied by RNA-RNA hybridization. With Wa and DS-1 (ss) RNA probes, human rotaviruses were found to separate into one of three categories. The majority of viruses (79%) shared significant homology with the Wa virus that belongs to subgroup 2 and serotype 2. Nineteen percent of viruses exhibited significant homology to the DS-1 virus that belongs to subgroup 1, serotype 1. Viruses belonging to the former group did not exhibit significant homology with the DS-1 virus or other viruses homologous to DS-1. Similarly, DS-1 related viruses showed little homology to Wa virus and its related viruses. Thus, 2 nonoverlapping human rotavirus families were identified. Of interest, 2% of human rotaviruses examined did not belong to either family, suggesting that one or more additional human rotavirus families exist. Finally, evidence for genetic reassortment between the two major families was observed (Flores).

Molecular Cloning of Rotavirus Genes - In order to define genetic diversity at a more basic level and at the same time probe rotavirus structure and transcriptional control in greater depth, we initiated efforts to clone the genes of 6 different rotaviruses in *E. coli* using plasmid pBR322. Several features of the rotavirus genome are relevant to the strategy used for cloning. These are: (a) the segmented nature of the genome, (b) the fact that the genes are double stranded RNA, (c) the ability of the virus to synthesize mRNA *in vitro*, and (d) the lack of poly A at the termini of single-stranded (ss) RNAs transcribed *in vitro* and genomic double-stranded (ds) RNAs present in the virion. Based on those features, we used the following strategy for cloning rotavirus genes: (1) polyadenylation of ss and ds rotavirus RNA, (2) reverse transcription, (3) second strand synthesis, (4) tailing and hybridization to plasmid pBR322, and (5) transformation of *E. coli*.

ss as well as ds rotavirus RNAs were used as templates to synthesize cDNA. The ss RNAs were produced by *in vitro* transcription of highly purified rotavirus cores, while the ds RNAs weres obtained by phenol extraction of rotavirus particles. Poly A was added to the 3' ends of the RNAs using *E. coli* poly A polymerase. This provided a template for the oligo dT primer required for reverse transcription. The efficiency and completion of the reverse transcription achieved by using AMV reverse transcriptase was much higher when ss RNA was used as substrate, hence cDNA was produced from ss RNA in most experiments. (ss) DNA copies were obtained from several rotaviruses, including two calf strains (NCDV and UK), a human strain (Wa), a canine strain (CU-1), a porcine strain (OSU) and a simian strain Rh2). Electrophoretic analysis of the ss cDNA obtained from each of these strains revealed complete and approximately equimolar synthesis of DNA from each of the rotavirus genes (Flores).

AMV reverse transcriptase was also used for second strand synthesis after tailing the ss DNA with oligo dC (using terminal transferase) and priming the reaction with oligo dG, a procedure that yielded complete copies of ds cDNA since it avoided the subsequent use of S-1 nuclease. ds cDNA produced in this manner was again tailed (oligo dC) with terminal transferase and hybridized to plasmid pBR322 that had been previously digested with Pst 1 and tailed (oligo dG) with terminal transferase. The hybrid pBR322-rotavirus cDNA was used to transform *E. coli* HB 101 cells in the presence of tetracycline. Several hundred transformants were obtained from the various viruses used. A majority of the more than 300 transformants derived from NCDV contain NCDV DNA as determined by colony hybridization. At least 8 of the 11 rotavirus genes appeared to have been successfully cloned in this initial effort (Flores).

Cultivation and Antigenic Diversity of Human Rotaviruses - The initial assessment of antigenic diversity was made using human-bovine virus reassortants that exhibited neutralization specificity of the human parent. From 50 stool specimens, 35 human rotaviruses were rescued by coinfection with a *ts* mutant of bovine rotavirus. Each of the 35 viruses so rescued exhibited the neutralization specificity of a human virus. Analysis of the 35 reassortants indicated that the majority were similar to Wa virus, a prototype of serotype 2. The next most common serotype encountered consisted of viruses that were similar in neutralization specificity to the simian rotaviruses, Sa-11 virus and the rhesus rotaviruses. Fewer viruses belonged to the third serotype that was typified by the DS-1 virus. Of interest, at least one additional serotype was detected among the 35 rescued human rotaviruses (Greenberg, Wyatt).

Recently Japanese investigators described conditions that permit direct cultivation of human rotaviruses in tissue culture. These include pretreatment of virus with trypsin, addition of trypsin to tissue culture medium and the rolling of inoculated tissue cultures. Using these modifications 32 human rotaviruses were isolated directly in tissue culture. Each of the 18 viruses tested thus far fit into one of the 4 serotypes described above. Thus it appears that antigenic diversity does not extend beyond 4 serotypes. Ten human rotaviruses were recovered directly in AGMK cells, a substrate satisfactory for production of vaccines destined for use in man (Wyatt).

Rotavirus Immunity as Studied in Experimental Animals - Experimental animals were studied under gnotobiotic conditions or in isolation to evaluate virulence of selected human and animal rotavirus strains as well as to study homologous and heterologous immunity. Gnotobiotic piglets were studied although gnotobiotic calves had been used extensively in the past. In a previous study of heterologous immunity, we demonstrated that *in utero* inoculation with bovine rotavirus protected calves against challenge with human rotavirus of a different serotype. Recent reevaluation of sera from calves in this study indicated that a single exposure to bovine rotavirus induced broadly reactive neutralizing antibody, which may explain the observed heterologous immunity. In a study of homologous immunity, piglets were exposed to human rotavirus "Wa" *in utero* and again at birth. Control animals without prior exposure to rotavirus shed significantly more virus and tended to have more diarrhea than those inoculated with virus *in utero*. In an additional study piglets were inoculated post-natally with rhesus rotavirus and subsequently challenged with a human rotavirus ("M" strain) to look for immunity between these related strains. Prior rotavirus infection diminished viral shedding when piglets were challenged with human rotavirus ("M" strain). Experimental animal systems now exist for the evaluation of cross-immunity between rotavirus strains, and these studies should aid in the selection of appropriate vaccine strains (Wyatt, Oshino).

Evaluation of a Candidate Rotavirus Vaccine Strain in Volunteers - Previously we observed that a wild type human rotavirus (strain D of serotype 2) induced diarrheal illness in 5 of 18 adult volunteers. During this study serologic correlates of resistance were identified. Following adaptation of the Wa strain of human rotavirus (also serotype 2) to tissue culture we used this information to identify susceptible individuals so that we could investigate virulence of the tissue culture-adapted Wa mutant for adult volunteers. Six of 10 susceptible volunteers (i.e., individuals with a low level of serum antibody) were infected but they did not become ill. This suggested that the Wa rotavirus mutant was attenuated compared to the wild type D rotavirus previously studied in adult volunteers. These findings are encouraging with respect to the prospects for developing an effective live rotavirus vaccine. Unfortunately, three of the Wa volunteers developed a mild transaminase elevation 10 days post challenge; this response was transient and did not appear to be related to rotavirus infection. These 3 individuals again developed a mild transaminase elevation several weeks later. None of these individuals developed overt liver disease. Chimpanzees and rhesus monkeys also developed a mild transient rise in transaminase 8 to 11 days after inoculation with the tissue culture grown Wa inoculum. The cause of the transaminase elevations is not clear at this time. Currently, 4 other rotaviruses are being evaluated for their capacity to induce transaminase elevation in primates. Those strains that do not induce this response will be considered for subsequent study in volunteers (Wyatt, Kapikian, Chanock).

Honors and Awards

Robert M. Chanock

Awarded the Robert Koch Medal of the Robert Koch Foundation by Antje Huber, Minister for Youth, Family and Health, Federal Republic of Germany, Bonn, December, 1981.

Participant, Symposium on Viral Diseases in South-East Asia and the Western Pacific, sponsored by the World Health Organization, Australian Academy of Science, and Australian Society for Microbiology, Inc. Presented two papers, "Vaccines - New Developments," and "Respiratory Viruses - An Overview," and served as chairman of symposium on hepatitis viruses, Canberra, Australia, January 1982.

Invited speaker, Annual Meeting of Association of Teachers of Preventive Medicine, Bethesda, Md., April, 1982.

Albert Z. Kapikian

Poster session on "Studies of Rotavirus by IAHA: Distinctions of IAHA and Neutralization Antigens", on August 2, 1981 at 5th International Congress of Virology, Strasbourg, France.

Co-chairman of session on Viral Gastroenteritis on November 11, 1981, 15th Joint Working Conference on Viral Diseases, U.S.-Japan Cooperative Medical Science Program, Bethesda, Maryland, November 9-11, 1981.

Invited to make presentation on "The Viral Agents" at Symposium on "Gastroenteritis" on November 18, 1981. The Annual Meeting of the American Society of Tropical Medicine and Hygiene, in San Juan, Puerto Rico.

Invited to present a review paper on "Prospects for development of vaccine against rotavirus infection," at the second meeting of the Scientific Working group on Microbiology, Epidemiology, Immunology and Vaccine Development in Viral Diarrheas, held in Geneva, Switzerland, February 1-3, 1982. Dr. Kapikian is a member of the Scientific Working Group.

Invited to participate in meeting of the Steering Committee of the Scientific Working Group on Microbiology, Epidemiology, Immunology and Vaccine Development in Viral Diarrheas, in Geneva, Switzerland, February 4-5, 1982. Dr. Kapikian is a member of the Steering Committee.

Invited to make a presentation on "Prospects for Development of a Rotavirus Vaccine," on May 27, 1982, at a Symposium on Biological and Genetic and Immunologic Aspects of Enteropathogens," in Mexico City, May 26-28, 1982.

Poster session on "Studies of Rotaviruses by IAHA: Distinctions of IAHA and Neutralization Antigen," on May 27, 1982, at a Symposium on Biological and Genetic and Immunologic Aspects of Enteropathogens," in Mexico City, May 26-28, 1982.

Co-Chairman of session on Viral Gastroenteritis at U.S.-Japan Cooperative Medical Science Program; Working Conference on Rabies, Arboviruses including Dengue, Korean Hemorrhagic Fever and Viral Gastroenteritis. July 19-21, Tokyo, Japan.

Presentation on "Studies of human rotavirus in adult volunteers: induction of illness and correlates of resistance" at U.S.-Japan Cooperative Medical Science Program Working Conference on Rabies, Arboviruses including Dengue, Korean Hemorrhagic Fever and Viral Gastroenteritis. July 19-21, Tokyo, Japan.

Member of United States Panel on Viral Diseases of the U.S.-Japan Cooperative Medical Science Program, 1978-present.

Robert H. Purcell

Invited speaker, Symposium on Hepatitis B Virus sponsored by the Government of Taipei, Taiwan, November 1981.

Invited speaker, Third U.S.-Japan Joint Hepatitis Panel Conference, Washington, D.C., March, 1982.

Invited participant, Workshop on Hepatitis A Virus, Walter Reed Army Institute of Research, March, 1982.

Visiting Professor, Southwestern Medical School, Dallas, Texas, March, 1982.

Invited speaker, Annual Meeting of Association of Teachers of Preventive Medicine, Bethesda, Md., April, 1982.

Invited participant, Workshop on Hepatitis B Virus and Hepatocellular Carcinoma, Bethesda, Md., May, 1982.

Invited participant, World Health Organization Committee on Viral Hepatitis, Munich, Germany, May, 1982.

Richard G. Wyatt

Invited discussant, Molecular Approaches to Virus Diseases, a symposium in honor of Dr. Horace Hodes, held at Mt. Sinai School of Medicine, New York, June 14, 1981. Participated in panel discussion of "Infections Caused by Enteric Viruses."

Invited speaker, Workshop on Viral Diarrhea, Fifth International Congress of Virology, Strasbourg, France. August 2-7, 1981. Presented paper entitled, "*In Vitro* Cultivation and Characterization of Human Rotavirus."

Invited speaker, Fifteenth Joint Working Conference on Viral Diseases, U.S.-Japan Cooperative Medical Science Program, Bethesda, Md., November 9-11, 1981. Presented talk entitled, "Definition of Human Rotavirus Serotypes by Plaque Reduction Assay."

Invited speaker, Seminar on Recent Studies of Rotavirus Epidemiology, Serology and Biochemistry, American Society for Microbiology, Atlanta, Ga., March 7-12, 1982. Presented talk entitled, "Serotypic Diversity of Human Rotavirus," on March 10, 1982.

Invited lecturer, University of Maryland course MICB 420: Epidemiology and Public Health. Presented lecture on "Epidemiology of Viral Gastroenteritis," on April 27, 1982.

Invited speaker, Sixteenth Joint Working Conference on Viral Diseases. U.S.-Japan Cooperative Medical Science Program, Tokyo, Japan, July 19-21, 1982. Presented talks entitled, "Serotypic Characterization of Human and Animal Rotaviruses" and "Experimental Studies in Animals with Various Rotaviruses."

Speaker, First Annual Meeting, American Society for Virology, Cornell University, August 2-5, 1982. Presented talk entitled "Serotyping of human rotaviruses."

Invited speaker, Seventh International Symposium on Intestinal Microecology, Boston, Ma., August 7-8, 1982. Presented talk on "Development of Vaccines Against Rotaviral Disease;" moderated session on Intestinal Virology.

Ching-Juh Lai

Invited speaker, Symposium on Genetic Engineering: Recent Developments, January 11-13, 1982. Taipei, Taiwan.

Invited speaker, *In Vitro* Mutagenesis, May 12-16, 1982. Cold Spring Harbor, New York.

Participant, Gordon Research Conferences on ANimal Cells and Viruses. June 21-15, 1982. Tilton, New Hampshire.

Recipient of the NIAID Cash Award 1982.

Brian R. Murphy

Invited speaker, Vaccination Against Influenza; T or B Cell Immunity? Oxford, England, March, 1982.

Harry B. Greenberg

Invited speaker, U.S.-Japan Cooperative Medical Science Program, Viral Disease Panel, 1981.

Chairman, ASM panel on Rotavirus, March, 1982.

Member, Vice Chairman, NIAID Clinical Research Subpanel, July 1982-September 1982. The subpanel has the responsibility to review clinical research protocals of the Institute as a part of the assessment of risks and benefits as well as ethical issues involved in the protection of human subjects.

Anthony R. Kalica

Member of Editorial Board, Journal of Clinical Microbiology, ASM, 1982 to 1984.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00308-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

IN VITRO STUDIES OF HEPATITIS A VIRUS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Richard J. Daemer, Ph.D.	Microbiologist	LID	NIAID
OTHERS:	Stephen M. Feinstone, M.D.	Medical Officer	LID	NIAID
	Robert H. Purcell, M.D.	Head, Hepatitis Virology Section	LID	NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION
NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.7	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Hepatitis A virus has been successfully adapted to growth in African green monkey kidney tissue culture. Over 20 serial passages have been achieved, and infectivity titers as high as 10^8 infectious units per ml of cell concentrate have been measured. The virus is predominantly cell-associated and does not produce CPE. It was attenuated for chimpanzees after 10 serial tissue culture passages; reevaluation of the virus in chimpanzees after 20 tissue culture passages is in progress.

PHS-6040
(Rev. 10-76)

[illegible]
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00309-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

THE BIOLOGY OF HEPATITIS A VIRUS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Stephen M. Feinstone, M.D.	Medical Officer	LID	NIAID
OTHERS:	Richard J. Daemer, Ph.D.	Microbiologist	LID	NIAID
	Robert H. Purcell, M.D.	Head, Hepatitis Viruses Section	LID	NIAID

COOPERATING UNITS (if any)

Fairfield Hospital, Melbourne, Australia (Dr. I. Gust); University of North Carolina (Dr. M. Sobsey)

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HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION
NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.4	OTHER: 0.6

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have successfully isolated a strain of hepatitis A virus in African green monkey kidney tissue culture, a cell substrate suitable for vaccine development. Growth of the agent in vitro has been characterized and attenuation for chimpanzees documented. The strain of virus appears to have considerable potential for vaccine development.

PHS-6040
(Rev. 10-76)

PROJECT NUMBER (Do NOT use this space)

HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

Z01 AI 00321-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

MOLECULAR VIROLOGY OF HEPATITIS A VIRUS (HAV)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John Ticehurst, M.D.	Medical Officer	LID	NIAID
OTHERS:	Stephen M. Feinstone, M.D.	Medical Officer	LID	NIAID
	Richard J. Daemer, Ph.D	Microbiologist	LID	NIAID
	Robert H. Purcell, M.D.	Head, Hepatitis Viruses Section	LID	NIAID

COOPERATING UNITS (if any)

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LABORATORY OF INFECTIOUS DISEASES

SECTION
HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION
NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: 1.6	PROFESSIONAL: 0.6	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Attempts to clone hepatitis A viral RNA are in progress. Several clones have been produced, and these are being evaluated for specificity. A limiting factor in the study, requiring modification of procedures, is the exceedingly small quantity of HAV RNA available.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00310-01 LID

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

HYBRIDOMA ANTIBODIES TO PATHOGENIC VIRUSES

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Stephen M. Feinstone, M.D.	Medical Officer	LID	NIAID
OTHERS:	Hanson Koo, M.D.	Visiting Fellow	LID	NIAID
	Vincent J. McAuliffe, M.D.	Medical Officer	LID	NIAID
	Robert H. Purcell, M.D.	Head, Hepatitis Viruses Section	LID	NIAID

COOPERATING UNITS (if any)

NIAID, NIH, Bethesda, Maryland (Dr. A. Fauci)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
HEPATITIS VIRUSES SECTION

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NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: 1.6	PROFESSIONAL: 1.6	OTHER: 0.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Attempts to produce monoclonal antibody to hepatitis A virus are in progress. The development of new serologic tests for detection of monoclonal anti-HAV will simplify the procedure. In addition, we are attempting to produce chimpanzee-mouse hybridomas, using chimpanzees convalescent from experimental HAV infection.

PHS-6040
(Rev. 10-76)

PROJECT NUMBER (Do NOT use this space)	HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 AI 00311-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

SEARCH FOR NEW HEPATITIS AGENTS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert H. Purcell, M.D.	Head, Hepatitis Viruses	LID	NIAID
OTHERS:	Tomoteru Kamimura, M.D.	Guest Worker	LID	NIAID

COOPERATING UNITS (if any) Clinical Center Blood Bank (Dr. H. Alter); National Institute of Virology, Pune, India (Dr. K. Pavri); Medical College of Srinagar, India (Dr. M. S. Khuroo); Mt. Sinai Hospital Dr. H. Popper), Centers for Disease Control, Phoenix, Az. (Dr. J. Maynard); Polio Inst., Moscow, Russia (Dr. M. Balayan).

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LABORATORY OF INFECTIOUS DISEASES

SECTION
HEPATITIS VIRUSES SECTION

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NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.2	OTHER: 0.8

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

New hepatitis agents continue to be recognized. Recently, a form of epidemic hepatitis occurring in India was found not to be caused by recognized hepatitis viruses. Attempts to transmit an agent from acute-phase clinical samples to primates are in progress. In addition, there is new evidence for a second serotype of hepatitis A virus. Attempts to identify and characterize the new agent, through collaborative studies, are in progress.

PHS-6040
(Rev. 10-76)

[illegible] PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00312-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

CLINICAL STUDIES OF HEPATITIS B VACCINES

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John Ticehurst, M. D.	Medical Officer	LID	NIAID
OTHERS:	Robert H. Purcell, M.D.	Head, Hepatitis Viruses Section	LID	NIAID

COOPERATING UNITS (if any)

Baylor School of Medicine (Dr. B. Hollinger); Scripps Institute (Dr. R. Lerner), Div. of Molecular Virology and Immunology, Georgetown U., Washington, D. C. (Dr. J. Gerin).

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LABORATORY OF INFECTIOUS DISEASES

SECTION
HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION
NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Clinical testing and characterization of plasma-derived hepatitis B vaccine prepared by the NIAID will soon be completed. The vaccine is highly immunogenic, safe and well tolerated. An efficacy trial of the vaccine in infants will begin soon in Asia. Second generation hepatitis B vaccines are currently under development. Approaches include the use of recombinant DNA-derived viral antigen and synthetic polypeptides. Preliminary results of the former are encouraging; preliminary results with the latter suggest that problems associated with immunogenicity must be overcome before synthetic polypeptide vaccines for hepatitis B will be practical.

PHS-6040
(Rev. 10-76)

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PROJECT NUMBER
Z01 AI 00313-01 LID

PERIOD COVERED
October 1, 1982 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

MOLECULAR BIOLOGY OF HEPATOCELLULAR CARCINOMA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Richard J. Daemer, Ph.D.	Microbiologist	LID	NIAID
OTHERS:	Yu-Mei Wen, M.D.	Visiting Fellow	LID	NIAID
	Robert H. Purcell, M.D.	Head, Hepatitis Viruses Section	LID	NIAID

COOPERATING UNITS (if any)

Bureau of Biologics, Bethesda, Maryland (Dr. B. Merchant)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION
NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.8	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Many tumor-bearing animals develop antibodies to unique antigens associated with the oncogenic virus causing the tumor. These antigens, called "neoantigens," have been found in tumors caused by papovaviruses, adenoviruses, and herpes viruses. Hepatitis B virus, a hepadnavirus with suspected oncogenic potential cannot be transmitted to non-primates but patients with HBV-associated hepatoma might be expected to have antibody to a HBV-associated neoantigen if one exists. Using a hepatoma cell line that contains integrated HBV DNA, we have sought immunofluorescent antibody in sera of hepatoma patients. Approximately seven percent of sera from HBsAg-positive hepatoma patients contain an antibody that reacts with a nuclear antigen in the hepatoma cell line. This antigen has been found in another hepatoma cell line that also contains integrated HBV genome but not in three other hepatoma cell lines lacking HBV genome. The antigen is being further characterized to determine if it is the product of a transforming gene.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00314-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

WOODCHUCK HEPATITIS VIRUS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert Purcell, M.D.	Head, Hepatitis Viruses	LID	NIAID
OTHERS:	Antonio Ponzetto, M.D.	Expert	LID	NIAID

COOPERATING UNITS (if any)
Div. of Molecular Virology & Immunology, Georgetown U., Washington, D.C., (Dr. J. Gerin); New York State College of Veterinary Medicine (Dr. B. Tennant)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION
NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.7	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The woodchuck hepatitis virus (WHV) is taxonomically and serologically related to the hepatitis B virus. Infection with each of these viruses is associated with acute and chronic hepatitis and hepatic cell carcinoma in their respective hosts; these associations appear to be etiological in nature. The WHV/woodchuck model system provides a convenient means of studying the relationship between virus and host in the oncogenic process. Tests specific for the WHV antigen-antibody systems have been developed. They are being applied to a prospective study of WHV infection in newborn woodchucks and the ability of active immunization to prevent hepatic cell carcinoma. The results of the study should have important prognostic value in evaluating active immunoprophylaxis of hepatitis B virus in man.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00315-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

SEARCH FOR NEW HEPADNAVIRUSES

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John Ticehurst, M.D.	Medical Officer	LID	NIAID
OTHERS:	Robert H. Purcell, M.D.	Head, Hepatitis Viruses Section	LID	NIAID

COOPERATING UNITS (if any)
Veterinary Resources Branch, NIH, (Dr. R. Whitney)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION
NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Viruses similar to hepatitis B virus ("hepadnaviruses") have been identified in three non-human species: The Eastern woodchuck, the Beechey ground squirrel and the Pekin duck. It is likely that many other species harbor similar viruses. The existing animal hosts are of limited value because inbred strains suitable for detailed immunological studies are not available. We are searching for hepadnaviruses among inbred strains of rodents, especially those with a known high incidence of hepatoma, in hopes of finding a more useful animal model system. Sensitive assays of hepadnavirus infection have been modified to permit testing of the small quantities of serum available.

PHS-6040
(Rev. 10-76)

PROJECT NUMBER (Do NOT use this space)

HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

Z01 AI 00316-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

THE DELTA AGENT

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert H. Purcell, M.D.	Head, Hepatitis Viruses	LID	NIAID
OTHERS:	Antonio Ponzetto, M.D.	Expert	LID	NIAID
	Tomoteru Kamimura, M.D.	Guest Worker	LID	NIAID

COOPERATING UNITS (if any) Div. of Molecular Virology & Immunology, Georgetown U., Washington, D.C., (Dr. J. Gerin); Centers for Disease Control, Phoenix, Ariz. (Dr. S. Hadler); Mt. Sinai Hospital, N.Y.C. (Dr. H. Popper).

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION
NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.7	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline key-words)

The delta agent is a transmissible hepatitis agent that appears to be defective and requires co-infection with hepatitis B virus for its own synthesis. The agent was discovered in 1977 in Italy, where it is endemic. Evidence for infection with the delta agent is found most frequently in carriers of hepatitis B virus who are repeatedly exposed to blood (hemophiliacs, illicit drug users, etc.). Sensitive assays for delta agent infection have been developed and used to evaluate experimental infection of HBV-carrier chimpanzees. In both man and chimpanzee infection with the delta agent results in very severe hepatitis. The delta agent has been experimentally transmitted to woodchucks chronically infected with the woodchuck hepatitis virus, a hepatitis virus similar to hepatitis B virus. The chimpanzee and woodchuck provide animal model systems for more detailed characterization of this medically important agent.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00317-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

BIOLOGY OF NON-A, NON-B HEPATITIS AGENTS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Stephen M. Feinstone, M.D.	Medical Officer	LID	NIAID
OTHERS:	Robert H. Purcell, M.D.	Head, Hepatitis Viruses Section	LID	NIAID

COOPERATING UNITS (if any)

Clinical Center Blood Bank, NIH (Dr. H. Alter and Dr. H. Shiraishi)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION
NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.3	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Although non-A, non-B hepatitis agents cannot be detected by serologic means, they can be experimentally transmitted to chimpanzees and marmosets. These species have been useful in determining the infectivity titers of various non-A, non-B containing plasmas. Although most plasmas contain only 10^2-10^3 infectious units per ml, one plasma was found to contain over 10^6 infectious units per ml. This plasma has provided an inoculum suitable for characterization of the agent. We have recently demonstrated that at least one non-A, non-B agent contains essential lipids, a characteristic that will be important in the classification and, probably, the control of non-A, non-B hepatitis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00318-01 LID

PERIOD COVERED
October 31, 1981 through September 31, 1982

TITLE OF PROJECT (80 characters or less)

MOLECULAR BIOLOGY OF NON-A, NON-B HEPATITIS AGENTS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert H. Purcell, M.D.	Head, Hepatitis Viruses	LID	NIAID
OTHERS:	Stephen M. Feinstone, M.D.	Medical Officer	LID	NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION
NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.4	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Attempts to identify non-A, non-B hepatitis agents by serologic means have been uniformly unsuccessful throughout the world. We have sought to apply recent advances in nucleic acid chemistry to a search for the genome of the non-A, non-B agent. Sensitive radiolabeling procedures have been modified to permit labeling of minute quantities of nucleic acid. Identification of the genome of the non-A, non-B agent would permit its characterization and cloning.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00319-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

NEW APPROACHES TO THE IN VITRO PROPAGATION OF NON-CULTIVATABLE AGENTS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Richard J. Daemer, Ph.D.	Microbiologist	LID	NIAID
OTHERS:	Robert H. Purcell, M.D.	Head, Hepatitis Viruses Section	LID	NIAID

COOPERATING UNITS (if any)

Johns Hopkins University Medical School, Baltimore, Md. (Dr. L. Widman)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION
NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline key-words)

There is a need for an in vitro substrate for the cultivation of hepatitis viruses. Hepatocytes would seem a logical choice, but it is very difficult to obtain and maintain primate hepatocytes in culture. We are attempting to develop hepatocyte-hepatoma hybridomas of primate origin. Such hybrid cells would be expected to have the receptor sites and metabolic systems suitable for synthesis of hepatitis viruses and the ability of hepatoma cells to multiply indefinitely in vitro.

PHS-6040
(Rev. 10-76)

PROJECT NUMBER (Do NOT use this space)

HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

Z01 AI 00320-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

PARVOVIRUS-LIKE AGENTS AND HEMATOLOGICAL DISORDERS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert H. Purcell, M.D.	Head, Hepatitis Viruses	LID NIAID
OTHERS:	Philip Mortimer, M.D.	Guest Worker	LID NIAID

COOPERATING UNITS (if any)

NHLBI, Bethesda, Maryland (Dr. N. Young); Children's Hospital, Washington, D.C.

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION
NIAID, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Severe hypoplastic crisis among patients with sickle cell anemia has been associated with infection with what appears to be a non-cultivatable parvovirus-like agent. The presumed agent was first identified by the relatively insensitive counterelectrophoresis technique. The immune adherence hemagglutination technique has been adapted to identification of this agent and its antibody: approximately 60 percent of normal populations have been found to be antibody-positive. Attempts to transmit the serum parvovirus-like agent to primates and to isolate it in primary cultures of human bone marrow are in progress.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00322-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

MOLECULAR BIOLOGY OF RESPIRATORY SYNCYTIAL VIRUS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Venkatesan, M.D.	Expert	LID NIAID
OTHERS:	N. Elango, Ph.D.	Visiting Fellow	LID NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
RESPIRATORY VIRUSES SECTION

INSTITUTE AND LOCATION
NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Efforts in the RS virus genetics program were redirected to concentrate on the molecular characterization of the viral genome and its products. A cDNA library of RS virus mRNA (from infected cells) was constructed in E. coli. using a plasmid vector and oligo dG/dC tailing. The first cDNA was transcribed by a technique that avoided the use of S1 nuclease and self priming. Recombinant plasmids containing viral sequences for at least 4 viral proteins were identified by (a) selection of plasmid DNAs that reacted with viral specific labeled mRNA, (b) hybrid selection of mRNA by recombinant cDNAs and translation of such mRNAs and/or (c) hybrid arrested translation of viral mRNAs by recombinant plasmids. The cloned viral DNAs in these plasmids are currently being sequenced to establish the reading frame of the various viral polypeptides.

PHS-6040
(Rev. 10-76)

(Do NOT use this space)

HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00323-01 LID

PERIOD COVERED
October 1,1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

PNEUMOVIRUS AND PARAMYXOVIRUS SURFACE GLYCOPROTEINS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Venketasan, M.D.	Expert	LID	NIAID
OTHERS:	N. Elango, Ph.D.	Visiting Fellow	LID	NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
RESPIRATORY VIRUSES SECTION

INSTITUTE AND LOCATION
NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our interest in parainfluenza and RS virus surface glycoproteins stems from the critical role they play in virus attachment, virus penetration, host range and tissue specificity. Equally important is the critical role these surface polypeptides play in the induction of immunity to infection and resistance to disease. To date, clones representing four non-surface antigen genes have been identified -- nucleoprotein (44kd), matrix protein (28kd), phosphoprotein (33kd), a non-structural protein (17kd). Characterization and sequencing of the cloned genes is in progress. Although cloned DNA representing the viral glycoproteins has not been identified, it should be possible to produce the desired glycoprotein DNA clones by "walking across" the viral RNA genome (i.e., using cloned DNA as a primer to extend reverse transcription of DNA sequences into an adjacent region of the genome). Optimism for this approach is based upon the fact that the gene order for parainfluenza viruses appears to be 5' NP, P, Fo, M., HN 3'. If RS virus has the same gene order, clones coding for the M and P genes could be extended into the adjacent genes on the negative-strand template and subsequently amplified by cloning in *E. coli*.

PHS-6040
(Rev. 10 76)

[illegible]
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00344-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

IMMUNOLOGIC CHARACTERIZATION OF RESPIRATORY SYNCYTIAL VIRUSES

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Gregory Prince, D.D.S., Ph.D.	Expert	LID	NIAID
OTHERS:	Robert M. Chanock, M.D.	Chief	LID	NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
RESPIRATORY VIRUSES SECTION

INSTITUTE AND LOCATION
NIAID, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.3	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline key-words)

Earlier efforts to develop a vaccine to RS virus dwelt primarily on the alteration of wild-type human RS (HRS) virus. To date, none of these efforts has been successful. The discovery of anaturally-occurring bovine RS (BRS) virus, with only limited similarity to HRS virus, led us to test wild-type BRS virus as a potential human vaccine. Bovine RS (BRS) virus was investigated for its capacity to induce resistance against human RS(HRS) virus in cotton rats, a permissive host for both viruses. Infection of cotton rats with BRS virus induced complete resistance in the lungs to HRS virus. Unfortunately, BRS virus is markedly restricted in chimpanzees and does not induce resistance to HRS virus.

A claim of significant antigenic variation among HRS viruses by other investigators could not be confirmed when a series of strains were compared by cross neutralization. Lack of significant antigenic difference between 2 strains was confirmed by cross challenge in cotton rats.

PHS-6040
(Rev. 10-76)

PROJECT NUMBER (Do NOT use this space)

HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00345-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

IMMUNITY TO RS VIRUS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Gregory Prince, D.D.S., Ph.D.	Expert	LID	NIAID
OTHERS:	Robert M. Chanock, M.D.	Chief	LID	NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
RESPIRATORY VIRUSES SECTION

INSTITUTE AND LOCATION
NIAID, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.3	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Previously we observed that active immunity to RS virus is of a different nature in the lungs than the nose, pulmonary immunity being more durable. The nature of active immunity was then studied using the technique of parabiosis. Parabiosis studies in cotton rats established that there is a systemic component of active immunity to RS virus. This systemic component provided resistance in the lungs but not the nose. Preliminary evidence suggests that serum antibodies play a role in this resistance.

Maternally-transmitted immunity to RS virus was also studied in cotton rats because a recent epidemiologic study of infants and children suggested that breast feeding conferred demonstrable resistance to serious RS virus respiratory tract disease during the first two years of life. We observed that passive immunity in cotton rats was transferred from the mother to her infants. This form of immunity was transient, lasting one week in the nose and 4 weeks in the lungs. Most of this type of immunity was transferred during the post natal period via colostrum and milk.

PHS-6040
(Rev. 10-76)

[illegible] PROJECT NUMBER (Do NOT use this space)	[illegible] OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00324-01 LID

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

LABORATORY STUDIES OF MYXOVIRUSES

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI.	Brian R. Murphy, M.D.	Medical Officer	LID	NIAID
OTHERS:	Robert M. Chanock, M.D.	Chief	LID	NIAID
	Shu-Fang Tian, M.D.	Fogarty Fellow	LID	NIAID
	Alicia Buckler-White, Ph.D.	Staff Fellow	LID	NIAID
	Judith Massicot, Ph.D.	Chemist	LID	NIAID
	Virginia Hinshaw, Ph.D.	St. Jude Children's Hospital		
	Robert Webster, Ph. D.	St. Jude Children's Hospital		
	Dorothy Holmes, D.V.M.	Cornell Univ. School of Medicine		
	Nicola Green, Ph.D.	Scripps Institute		
	Richard Lerner, Ph.D.	Scripps Institute		

COOPERATING UNITS (if any)

St. Jude Children's Hospital, Memphis, Tenn.; Cornell Univ. School of Vet. Medicine, Ithaca, N.Y.; Scripps Institute, La Jolla, California

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
RESPIRATORY VIRUSES SECTION

INSTITUTE AND LOCATION
NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.6	2.6	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A new ts donor virus that had been shown to be more stable in vivo and in vitro than the ts-1[E] and ts-1A2 donor viruses nonetheless lost its ts phenotype after in vitro stress at restrictive temperature. Its ts phenotype was also lost during replication in vivo in cyclophosphamide treated hamsters. For these reasons work with ts mutants has been terminated. A reassortant virus containing the hemagglutinin and neuraminidase genes of a virulent human virus and the six internal genes of an attenuated avian virus was produced. This reassortant was attenuated and immunogenic in the hamster. This avian-human reassortant virus lost its enterotropism for ducks but retained its bursal tropism. Additional avian-human reassortants were produced for use in the characterization of the genetic determinants of virulence of influenza A viruses for primates and for ducks. Synthetic peptides of the A/Victoria/75 (H3) hemagglutinin were administered to hamsters. The hamsters did not develop an HI antibody response and manifested only minimal resistance to wild type challenge.

PROJECT NUMBER (Do NOT use this space)

HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00325-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

STUDY OF RESPIRATORY VIRUSES IN PRIMATES

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI.	Brian R. Murphy, M.D.	Medical Officer	LID NIAID
OTHERS:	Robert M. Chanock, M.D.	Chief	LID NIAID
	David Sly, D.V.M.		Meloy Laboratories
	William London, D.V.M.		NINDS NIH

COOPERATING UNITS (if any)
Meloy Laboratories, Rockville, Maryland
NINDS, NIH

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
RESPIRATORY VIRUSES SECTION

INSTITUTE AND LOCATION
NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: 1.5	PROFESSIONAL: 0.5	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The reassortant virus containing the surface antigens of the A/Udorn/72 (H3N2) human virus and the "internal" six genes from an attenuated avian virus was evaluated for virulence in squirrel monkeys. The reassortant virus replicated to the same level as its avian parent and was fully attenuated in monkeys. These results indicated that the internal genes of the avian influenza A virus were the primary determinants of attenuation for the monkey. Squirrel monkeys infected with the reassortant virus were resistant to wild type virus challenge. This represents a new method for the production of a live influenza A vaccine virus in which attenuating avian genes are transfered to a human virus. The resulting reassortant virus is attenuated but immunogenic in primates (and, hopefully, man).

PROJECT NUMBER (Do NOT use this space)	HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00326-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

STUDY OF RESPIRATORY VIRUSES IN VOLUNTEERS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI.	Brian R. Murphy, M.D.	Medical Officer	LID NIAID
	Robert M. Chanock, M.D.	Chief	LID NIAID
	Louis Potash, Ph.D.		Flow Laboratories
	Marylou Clements, M.D.		University of Maryland
	Robert Betts, M.D.		University of Rochester
	Robert Yarchoan, M.D.		NCI NIH
	David Nelson, M.D.		NCI NIH
	Raphael Dolin, M.D.		University of Vermont
	Peter Wright, M.D.		Vanderbilt University
	Bruce Burlington, M.D.		Bureau of Biologics
	Michael Phalen		Bureau of Biologics

COOPERATING UNITS (if any) Flow Laboratories, Rockville, Md.; U. of Md. School of Med., Balto., Md., U. of Rochester, School of Med., Rochester, N.Y.; Nat. Cancer Inst., Immunology Branch, Bethesda, Md.; U. of Vermont, Burlington, Vt.; Vanderbilt U., Nashville, Tenn.; Bureau of Biologics, FDA, Bethesda, Md.

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
RESPIRATORY VIRUSES SECTION

INSTITUTE AND LOCATION
NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.1	0.6	3.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dose response of the A/Alaska/77 (H3N2) ca reassortant virus was completed this year. The human infectious dose_{50} was $10^{5.5}$ TCID_{50}. The presence of local nasal wash, IgA anti hemagglutinin antibody correlated with resistance to infection with vaccine virus. The A/HK/123/77 (H1N1) ca reassortant induced resistance to wild type virus infection in seronegative adults. The A/Cal/10/78 (H1N1) ca reassortant virus was attenuated, genetically stable, and non-transmissible in seronegative adults. The HID_{50} was about $10^{6.2}$ TCID_{50}. The A/Washington/897/80 (H3N2) ca virus was also attenuated in seronegative adults. Febrile illness occured when vaccinees were administered a dose of approximately $10^{7.5}$ TCID_{50} of ca virus but the frequency was usually ≤5% of susceptible vaccinees.

Immunological memory in the IgA isotype was demonstrated for the upper respiratory tract. During primary infection of adults serum antibody occurs in IgG, IgA, and IgM isotypes, but during secondary infection antibody occurs mostly in IgA and IgG isotypes. The magnitude of IgA antibody produced in the local respiratory tract correlates with that in serum of individuals infected with influenza A virus.

PROJECT NUMBER (Do NOT use this space)

HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00327-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

CHARACTERIZATION OF INFLUENZA A VIRUSES USING MONOCLONAL ANTIBODIES

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Kathleen L. van Wyke, Ph.D.	Senior Staff Fellow	LID	NIAID
OTHERS:	Brian R. Murphy, M.D.	Medical Officer	LID	NIAID

COOPERATING UNITS (if any)

St. Jude Children's Research Hospital, Memphis, Tennessee; Vincent Lombardi Center for Viral Oncology, Georgetown University, Washington, D. C.

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
RESPIRATORY VIRUSES SECTION

INSTITUTE AND LOCATION
NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Since monoclonal antibodies have been useful for the examination of antigenic and functional properties of several influenza virus proteins, we have utilized a similar approach to the study of influenza matrix and polymerase proteins. We generated five monoclonal antibodies that inhibit the in vitro RNA transcriptase activity of influenza virus and bind to a protein which co-migrates with the influenza polymerase proteins in acrylamide gels. Preliminary results indicate that these antibodies are directed at the polymerase enzyme(s) of influenza virus, but additional study is necessary to conclusively demonstrate their specificities.

Six monoclonal antibodies to the matrix protein are also under investigation. Preliminary results indicate that little antigenic variation occurs in the influenza virus matrix protein.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00328-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

REQUIREMENTS FOR CELL SURFACE EXPRESSION OF INFLUENZA A VIRUS HEMAGGLUTININ FROM CLONED FULL-LENGTH DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael M. Sveda, Ph.D.	Expert	LID	NIAID
OTHERS:	L. Markoff, M.D.	Medical Officer	LID	NIAID
	C.-J. Lai, Ph.D.	Head, MBV Section	LID	NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
MOLECULAR VIRAL BIOLOGY SECTION

INSTITUTE AND LOCATION
NIAID, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: 1.3	PROFESSIONAL: 1.0	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Requirements for cell surface expression of the influenza hemagglutinin (HA) were studied using a derived recombinant of SV40 which had full-length DNA sequences coding for the influenza virion HA inserted into the late region of SV40 DNA and propagated in the presence of tsA SV40 helper. Infection of primate cells with the HA-SV40 recombinant produced a functional glycosylated HA polypeptide that accumulated on the cell surface. To delineate the protein domains necessary for surface expression of the HA polypeptide, mutations were engineered in the recombinant HA-SV40 DNA. Deletion of the entire region coding for the hydrophobic COOH-terminus of the HA protein produced an HA polypeptide present first as a truncated glycosylated intracellular protein and later as a larger glycosylated secreted protein. The 2 proteins differed in their sensitivity to endoglycosidase H. A frameshift mutation at the beginning of the hydrophobic COOH-terminus introduced six charged amino acid residues. The resulting HA polypeptide was similar in size to and had the same endoglycosidase H sensitivity as unaltered HA. It was not secreted, was not on the cell surface, and was only present intracellularly. These findings suggest a role for the hydrophobic COOH-terminus not only in cell surface expression but for secretion related glycosylation.

PROJECT NUMBER (Do NOT use this space)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00329-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

STUDIES OF AN INFLUENZA A VIRUS NEURAMINIDASE GENE USING RECOMBINANT DNA TECHNIQUES

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Markoff, M.D.	Medical Officer	LID	NIAID
OTHERS:	C.-J. Lai, Ph.D.	Head, MVB Section	LID	NIAID
	Bor-Chian Lin, Ph.D.	Visiting Associate	LID	NIAID
	M. Sveda, Ph.D.	Expert	LID	NIAID

COOPERATING UNITS (if any)

St. Jude Childrens' Hospital, Memphis, Tennessee (Dr. Robert Webster)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
MOLECULAR VIRAL BIOLOGY SECTION

INSTITUTE AND LOCATION
NIAID, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: 1.3	PROFESSIONAL: 1.1	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An influenza A N2 neuraminidase (NA) gene was cloned at the Pst 1 site of pBR322 and completely sequenced. The resulting nucleotide sequence included the expected features of a complete copy of an influenza virion RNA. The deduced amino acid sequence of the N2 NA polypeptide was consistent with that determined for an NI NA gene sequence and suggested an amino terminal orientation for membrane insertion of the glycoprotein. To study expression of the NA gene in mammalian cells, a complete copy of the N2 NA gene was cloned in the Bam H1 site of pBR322 and then in the late region of SV40 virus. African Green Monkey kidney cells infected with this vector produced an immunoprecipitable polypeptide comparable in size to NA polypeptide produced during influenza virus infection and detectable at the cell surface by indirect immunofluorescence. Functional expression was not established. To study processing and maturation, deletion mutations were generated in separate experiments at unique restriction endonuclease sites near the carboxy or the amino terminus of the molecule. One deletion resulting in absence of the entire carboxy terminus of the molecule yielded a truncated immunoprecipitable polypeptide that was not secreted, implying that membrane anchorage occurred normally.

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PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

CLONING, EXPRESSION AND FUNCTIONAL DOMAINS OF THE INFLUENZA VIRUS GENE CODING FOR NUCLEOPROTEIN

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Bor-Chian Lin, Ph.D.	Visiting Associate,	LID	NIAID
	Ching-Juh Lai, Ph.D.	Head, MBV Section,	LID	NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
MOLECULAR VIRAL BIOLOGY SECTION

INSTITUTE AND LOCATION
NIAID, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline key-words) We obtained DNA sequences coding for the nucleoprotein (NP) of an influenza A virus by reverse-transcription of vRNA using synthetic oligonucleotide primers. Terminal sequence analysis showed that the cloned gene contained a full-length copy of the virion RNA segment. The NP specific DNA was inserted into the late region of an SV40 vector and the DNA recombinant was propagated in the presence of an early SV40 ts mutant helper. Infection of African green monkey kidney cells with the recombinant produced a polypeptide immunoprecipitable with NP-specific antisera. The polypeptide product had a molecular weight of 56K daltons identical to the nucleoprotein of influenza virus as estimated on polacrylamide gels. The putative NP was detected in the nucleus of infected primate cells by an immunofluorescence assay. This nuclear localization of NP from recombinant DNA was similar to that seen during influenza virus infection suggesting the NP product may be functionally active. To further delineate the functional domains of NP as defined by several classes of monoclonal antibodies, we have recently constructed deletion mutants of the NP gene and prepared recombinants of SV40-NP. These recombinants synthesized polypeptide products representing different regions of the full-length NP molecule. Experiments are under way to characterize the cellular location of these shortened NP polypeptides and their antibody binding affinities.

PHS-6040
(Rev. 10-76)

PROJECT NUMBER (Do NOT use this space)

HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

Z01 AI 00331-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

TRANSCRIPTIONAL CONTROL OF INFLUENZA A VIRUS: SYNTHESIS OF SPLICED AND UNSPLICED mRNAs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ching-Juh Lai, Ph.D.	Head, Molecular Viral Biology Sect.	LID NIAID
OTHERS:	Robert A. Lamb, Ph.D.	Associate Professor	Rockefeller U.

COOPERATING UNITS (if any)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
MOLECULAR VIRAL BIOLOGY SECTION

INSTITUTE AND LOCATION
NIAID, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.2	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Following infection of a permissive cell, influenza viral cytoplasmic mRNA's are transcribed from the virion RNA segments. We showed previously that both uninterrupted (colinear) and interrupted mRNA species are derived from viral RNA segments coding for the matrix protein (M) and non-structural proteins (NS). Two interrupted mRNAs, M2 mRNA and M3 mRNA, are made. To demonstrate that interrupted mRNA's are generated by splicing, cloned mDNA was inserted into an SV40 vector so that the late SV40 transcription signals direct the synthesis of (+) strand M RNA sequences. Poly A-containing RNA from African green monkey kidney cells infected with the SV40-M recombinant was analyzed for the presence of M sequences. The results showed that a colinear and an interrupted M RNA species were produced. The interrupted M RNA contained a splice sequence at the juncture similar to that of M3 mRNA found during influenza infection. Persuant to the mRNA analysis, we examined the M-specific polypeptide of the SV40-M recombinant by immunoprecipitation and gel electrophoresis. As predicted, the matrix protein (M_1) of influenza virus was detected. Specific splicing from the transcript of SV40-M DNA ruled out the possibility that influenza mRNA's are formed by polymerase "jumping" at the consensus sequences during replication. The presence of colinear as well as spliced M mRNA's establishes that processing occurs during in vivo influenza virus transcription.

PHS 6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00332-01 LID

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

ALLELE REPLACEMENT OF INFLUENZA VIRUS GENE SEGMENTS USING CLONED DNA SEQUENCES

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Ching-Juh Lai, Ph.D.	Head, MVB Section	LID	NIAID
Lewis Markoff, M.D.	Medical Officer	LID	NIAID
Michael Sveda, Ph.D.	Expert Consultant	LID	NIAID
Bor-Chian Lin, Ph.D.	Visiting Associate	LID	NIAID
Kenji Sekikawa, Ph.D.	Visiting Associate	LID	NIAID
Robert Chanock, M.D.	Chief	LID	NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
MOLECULAR VIRAL BIOLOGY SECTION

INSTITUTE AND LOCATION
NIAID, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: 1.7	PROFESSIONAL: 1.4	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We obtained cloned full-length DNA copies of influenza viral gene segments that code for the nonstructural proteins (NS), membrane protein (M), neuraminidase (NA), nucleoprotein (NP), hemagglutinin (HA), and polymerase protein (P3). Cloned complete DNA sequences were inserted into the late region of SV40 and the recombinant DNA was propagated in the presence of a tsA SV40 helper. SV40-influenza DNA recombinants that synthesize either the (+) or the (-) full-length strand of influenza viral transcripts were isolated for use in allele replacement, that is, conversion of cloned DNA back into virion RNA. As a first step, we employed the technique of "marker rescue" under antibody selection using the H1N1 subtype of an influenza virus (strain WSN) and an SV40-HA (H3) or SV40-NA (N2) recombinant that produced the (+) strand of influenza viral RNA transcripts in a coinfected cell. Similarly, "marker rescue" of an influenza ts mutant defective in P3 was tested with an SV40-P3 recombinant that transcribed the (+) strand of influenza viral P3 RNA. Preliminary characterization by immunologic techniques and/or gel analysis did not identify progeny that contained a substituted allele corresponding to a rescued viral gene and seven remaining genes from WSN virus. Experiments using recombinants that transcribe the (-) strand viral RNA are under way to determine whether this approach will prove successful for use in allele replacement.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00343-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

THE ROLE OF NORWALK-LIKE 27 nm VIRUS PARTICLES IN VIRAL GASTROENTERITIS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Harry Greenberg, M.D.	Medical Officer	LID NIAID
OTHERS:	Margaret A. Walsh, M.D.	Medical Staff Fellow	LID NIAID
	Karen Midthun, M. D.	Medical Staff Fellow	LID NIAID

COOPERATING UNITS (if any)

SEE ATTACHED PAGE

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
EPIDEMIOLOGY SECTION

INSTITUTE AND LOCATION
NIAID, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.4	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline key-words) A variety of 27 to 32 nm viruses have been shown to be etiologically associated with epidemic nonbacterial gastroenteritis. The Norwalk virus is the most thoroughly studied member of this group. Seroepidemiologic studies using a sensitive solid phase RIA indicate: (1) Norwalk virus has a world wide distribution, (2) infections occur at a younger age in less developed countries, (3) Norwalk infection is responsible for at least 1/3rd of outbreaks of nonbacterial gastroenteritis in the U.S., (4) the majority of outbreaks are waterborne but some are associated with contaminated food or shell fish, or cannot be associated with a specific source, and (5) in less developed countries Norwalk infection may be associated with mild gastroenteritis in young children. Attempts to propogate Norwalk and other small gastroenteritis viruses have not been successful but biophysical characterization of virus derived from feces indicates that its proteins most closely resemble those of caliciviruses. Recently a serologically distinct 27 nm gastroenteritis virus, the Marin County virus, was successfully transmitted to adult volunteers. One volunteer developed a diarrheal illness and shed large amounts of virus that was detected by immune electron microscopy. The Marin County virus has a buoyant density similar to Norwalk virus. Serologic assays will be developed to determine its importance in outbreaks of epidemic gastroenteritis.

Cooperating Units:

Calif. State Health Dept., Berkeley, Calif., (L. Oshiro); Dept. of Medicine, Michigan State U. (M. Gurwith); Bureau of Epidemiology, Washington, D. C. and Atlanta, Ga. (M. Griffin, R. Wilson, J. Kaplan, W. Gary, R. Gunn, R. Baron); Dept. of Medicine, VA Hosp., Minneapolis, Minn. (R. Gebhard); Dept. of Infec. Disease, U. of Vermont Medical School (D. Gump); Dept. of Health Services, State of Washington, (J. Taylor); Dept. of Geographic Medicine, Johns Hopkins University, Baltimore, Md. (M. Santoshan); Dept. of Epidemiology, U. of Michigan (J. Koopman); Univ. of Md. Medical School, Baltimore, Md. (R. Black).

PROJECT NUMBER (Do NOT use this space)

HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

Z01 AI 00333-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

A LONGITUDINAL STUDY OF VIRAL GASTROENTERITIS IN INFANTS AND YOUNG CHILDREN AND OTHER EPIDEMIOLOGICAL STUDIES

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Albert Z. Kapikian, M.D.	Head, Epidemiology Section	LID	NIAID

COOPERATING UNITS (if any)

Johns Hopkins Univ., Baltimore, Md.; ICDDR, Bangladesh, India; U. of Md.; Gorgas Memorial Laboratory, Panama

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
EPIDEMIOLOGY SECTION

INSTITUTE AND LOCATION
NIAID, BETHESDA, MARYLAND 20852

TOTAL MANYEARS: 1.15	PROFESSIONAL: .25	OTHER: 0.9

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Rotaviruses have been studied extensively by predominantly cross-sectional approaches. Such studies have yielded essentially "numerator" data which indicated that rotaviruses are a major cause of diarrheal illness. There have been few longitudinal gastroenteritis studies yielding important epidemiologic information. Therefore we initiated an examination of anal swab and serum specimens obtained during a previous long-term longitudinal study (1955-1969) at Junior Village, a welfare institution for normal, homeless children. Anal swabs and blood specimens were obtained routinely. Surveillance was carried out by a trained medical staff. Thus far, 139 rotavirus strains have been detected with the characteristic seasonal distribution. It should be possible to establish the serotypic diversity of these strains. The subgrouping pattern is consistent with other studies as 31 of 39 belong to subgroup 2. In addition, sequential sera from 384 children in residence sometime between May 19, 1963-May 31, 1966 have been tested for CF antibody to the "O" agent. 150 (40%) of the children experienced at least one rotavirus infection; in addition, 11 had a second infection and one a third infection. For the period from May 22, 1966-May 21, 1969 65 (36%) of 182 children (some overlap with previous period) experienced at least one rotavirus infection, with 6 having a second infection. IAHA serology has also been analyzed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00334-01 LID

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

DEVELOPMENT OF MONOCLONAL ANTIBODIES TO PROTEINS OF ROTAVIRUS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Harry Greenberg, M.D.	Medical Officer	LID	NIAID
OTHERS:	Nalini Singh, M.D.	Visiting Scientist	LID	NIAID
	Karin Midthun	Medical Staff Fellow	LID	NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
EPIDEMIOLOGY SECTION

INSTITUTE AND LOCATION
NIAID, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: 2.8	PROFESSIONAL: 1.5	OTHER: 1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In an attempt to clarify rotavirus immunology and as a means of investigating rotavirus protein structure and function we have isolated a series of monoclones directed at several rotavirus proteins. Monoclones were derived from mice immunized with the Wa strain of human rotavirus, the rhesus 2 strain of simian rotavirus and a human rotavirus (DS-1) reassortant (41-2). We studied 70 monoclones directed at 5 viral proteins. Monoclone production utilized standard techniques and assay systems including RIA, neutralization, immunoprecipitation and hemagglutination-inhibition (HI). The monoclones were characterized by the proteins to which they react: (1) Monoclones to the 42,000 dalton inner protein, the 6th gene product; two of these have subgroup specificity. (2) Monoclones to the 34,000 dalton surface glycoprotein, the 8th or 9th gene product; some of these neutralize the virus to high titer and some have HI activity. (3) Monoclones to the 82,000 dalton surface protein, the 4th gene product, the viral hemmaglutinin; these monoclones inhibit hemagglutination and neutralize to low titer. (4) Monoclones to the 28,000 dalton 10th gene product, the 3rd viral surface protein; one of these monoclones neutralizes infectivity and one has low level HI activity. (5). Two monoclones appear to react with either the 2nd or 3rd viral protein, an 88,000 dalton inner protein.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00335-01 LID

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

DEVELOPMENT AND PHENOTYPIC ANALYSIS OF ROTAVIRUS REASSORTANTS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI.	Harry Greenberg, M.D.	Medical Officer	LID NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
EPIDEMIOLOGY SECTION

INSTITUTE AND LOCATION
NIAID, BETHESDA, MARYLAND

TOTAL MANYEARS: 1.7	PROFESSIONAL: 0.4	OTHER: 1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Rotaviruses have a double stranded segmented RNA genome and hence undergo genetic reassortment at high frequency. We have produced a series of temperature sensitive mutants of both UK (bovine) and Rhesus rotavirus. We have used these ts mutants to isolate reassortant rotaviruses from the growth yield of cells coinfected with a variety of human rotaviruses and ts bovine rotavirus. These reassortants were used to ascertain the gene coding assignments for human rotavirus neutralization specificity (gene 8 or 9), subgroup specificity (gene 6) and growth restriction in tissue culture (gene 4). In addition the human-bovine rotavirus reassortants were used to define the serotypic diversity of human rotaviruses (four or more serotypes). In addition, we isolated a series of reassortants derived from the growth yield of cells coinfected with ts UK and ts rhesus or ts UK, and canine rotaviruses. We characterized the phenotype of these reassortants and showed that: (1) neutralization specificity and hemagglutination reassort independently and hence are coded for by different genes; and (2) protease enhanced viral growth co-segregates with viral hemagglutination and hence these properties are coded for by the same gene. An understanding of the specific functions of the genes of rotavirus may have important implications for vaccine development. In addition, reassortant viruses represent potential vaccine virus candidates.

PROJECT NUMBER (Do NOT use this space)	HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 AI 00336-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

IDENTIFICATION AND CHARACTERIZATION OF ROTAVIRUS GENE PRODUCTS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Anthony R. Kalica, Ph.D.	Research Microbiologist	LID	NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
EPIDEMIOLOGY SECTION

INSTITUTE AND LOCATION
NIAID, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.9	0.8	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have identified genes that code for specific rotaviral functions such as neutralization, hemagglutination, growth restriction and protease enhanced infectivity. The general approach involves identification of reproducible differences between two rotaviral strains with respect to one or more such functions. Reassortant viruses are generated by coinfection of cell cultures and then analyzed for functional differences that correlate with specific viral genes. This approach takes advantage of the segmented rotaviral genome that allows reassortment to occur during coinfection. Genetic analysis of the reassortants by polyacrylamide gel electrophoresis (PAGE) permitted us to identify the parental origin of each gene. Using this approach we demonstrated that the 8th or 9th gene (depending on the strain) of human rotavirus codes for the neutralization antigen and that the 6th genomic segment codes for the subgroup antigen. Recently we have identified the 4th RNA segment as coding for rotaviral hemagglutinin. This protein is activated by a protease and such activation is required for virus to be infectious. Thus the ability of rotaviruses to infect cells is multigenic involving the products of at least two different genes.

PHS-6040
(Rev. 10-76)

PROJECT NUMBER (Do NOT use this space)

HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

Z01 AI 00337-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

ROTAVIRAL PROTEINS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Anthony R. Kalica, Ph.D.	Res. Microbiol.	LID NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
EPIDEMIOLOGY SECTION

INSTITUTE AND LOCATION
NIAID, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In the past year we focused on identification of the number of rotaviral structural and nonstructural proteins . As many as 15 SA-11 proteins were identified and 10 of the 15 were structural. Less success was realized with some of the other animal rotaviruses as well as with one human strain (Wa), for which 10 to 12 proteins were identified. Six or seven of them were structural. Emphasis was placed on developing methods to increase the yield of cell cultures infected with the more fastidious human strains in order to obtain higher titered stocks for use in biochemical studies.

Additional efforts were made to develop methods of labeling rotaviral proteins in cell lysates with ^{35}S methionine. These lysates were used to identify specific proteins which were immunoprecipitated by monoclonal antibodies. In order to obtain infected cell lysates which were suitable for immunoprecipitation experiments with monoclonal antibodies we developed a modification of a published method which had been successful for similar studies with reoviral monoclones. The method produced labeling of all of the proteins found previously in infected MA104 cells labeled by one standard method.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00346-01.LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

GENETIC DIVERSITY OF ROTAVIRUSES STUDIED BY RNA-RNA HYBRIDIZATION

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Flores, M.D.	Expert Consultant	LID NIAID

COOPERATING UNITS (if any)

Hospital de los Ninos, Caracas, Venezuela (Dr. Perez, L. White)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
EPIDEMIOLOGY SECTION

INSTITUTE AND LOCATION
NIAID, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: 1.7	PROFESSIONAL: 0.6	OTHER: 1.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The genetic relatedness among several human and animal rotavirus strains has been studied by hybridization techniques in which genomic RNA of a virus is hybridized to single-stranded RNA labeled probes synthesized by *in vitro* transcription from other strains. Gel electrophoresis of the resulting hybrids and analysis of their nuclease resistance have given an indication of the degrees of homology between the strains studied. An initial series, in which 9 human rotavirus strains from Venezuela were examined, has now been followed by a study in which more than a hundred well characterized human rotavirus strains obtained throughout a complete year (1981) have been analyzed. The results obtained with both groups suggest the existence of two rotavirus "families." In hybridization studies with two serotypically distinct human rotaviruses, DS-1 and Wa, the majority of the strains (79%) hybridized significantly to the Wa probe but not the DS-1 probe. These hybridization findings have now been confirmed and complemented by quantitative analysis that further establishes the distinctness between the two prototype viruses. In such studies, it was found that only 17 percent of sequence homology exists between Wa and DS-1 viruses. In addition, that there was 60-100 percent homology between many of the isolates studied and one of the prototype viruses.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00338-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

CHARACTERIZATION OF THE ROTAVIRUS GENOME BY RECOMBINANT DNA TECHNIQUES

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Flores, M.D.	Expert Consultant	LID NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASE

SECTION
EPIDEMIOLOGY SECTION

INSTITUTE AND LOCATION
NIAID, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.4	OTHER: 0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Single-stranded (ss) as well as double stranded ds) rotavirus RNA were used as templates to synthesize cDNA. The ss RNA was produced by in vitro transcription, while the ds RNA was purified from rotavirus particles. Since neither ss nor ds rotavirus RNA are polyadenylated, tailing of the 3' ends was necessary prior to dDNA synthesis in order to create an adequate primer for reverse transcription. After polyadenylation and reverse transcription, we obtained ss cDNA copies of apparently full size from several rotavirus strains, including calf strains (NCDV and UK), human strain Wa, canine strain CU-1 and porcine strain OSU. Synthesis of the second strand was achieved after tailing ss cDNA with oligo dC and priming with oligo dG, a procedure that yields complete copies of ds cDNA, since it avoids the subsequent use of S1 nuclease. Ds cDNA produced in this manner was inserted into plasmid PBR 322 and the hybrid used to transform E. coli HB-101. A majority of 300 NCDN transformants studied by colony hybridization contained NCDV DNA. These positive clones are currently being analyzed to determine size and gene origin of the cDNA inserts. Gene amplification and potential expression of rotavirus in bacteria should allow us to analyze the structure and genomic organization of these viruses.

PROJECT NUMBER (Do NOT use this space)	HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00339-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

CULTIVATION AND SEROTYPIC CHARACTERIZATION OF HUMAN AND ANIMAL ROTAVIRUSES

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Richard G. Wyatt, M.D.	Medical Officer	LID	NIAID
OTHERS:	Yasutaka Hoshino, D.V.M.	Visiting Fellow	LID	NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
EPIDEMIOLOGY SECTION

INSTITUTE AND LOCATION
NIAID, BETHESDA, MARYLAND

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.3	1.3	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline key-words)

This project is designed to cultivate directly in cell culture a variety of human rotavirus strains from diverse geographical areas and populations in order to define antigenic differences and to identify and develop potential vaccine candidates. In the evaluation of virus strains we have employed a variety of cell types including MA104 and primary African green monkey kidney (AGMK) cells. The technique for cultivation used in this study was described recently by Japanese investigators. Over 30 strains of human rotaviruses have been cultivated in MA104 cells and 10 of these were reisolated in AGMK cells. Four distinct serotypes of human rotavirus have been identified and compared with each other. The serotypic characterization of the reassortant human-bovine rotaviruses previously studied in our laboratory and these direct isolates was in agreement. It is of particular interest that a rhesus, canine, and feline rotavirus are all similar if not identical to one of the human rotavirus serotypes. Finally, it is important to note that several potential candidate vaccine strains of different serotypes have been grown in a cell type suitable for vaccine production, and these now can be evaluated further for safety and efficacy.

PHS-6040
(Rev. 10-76)

PROJECT NUMBER (Do NOT use this space)

HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00340-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

EXPERIMENTAL STUDIES IN ANIMALS WITH VARIOUS ROTAVIRUSES

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Richard G. Wyatt, M.D.	Medical Officer	LID NIAID
OTHERS:	Yasutaka Hoshino, D.V.M.	Visiting Fellow	LID NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
EPIDEMIOLOGY SECTION

INSTITUTE AND LOCATION
NIAID, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline key-words)

Experimental animals were studied under gnotobiotic conditions or in isolation to evaluate virulence of selected human and animal rotavirus strains as well as to study homologous and heterologous immunity. Gnotobiotic piglets were studied although gnotobiotic calves had been used extensively in the past. In a previous study of heterologous immunity, we demonstrated that in utero inoculation with bovine rotavirus protected calves against challenge with human rotavirus of a distinct serotype. Recent reevaluation of the calf sera from this study indicated that a single exposure to bovine rotavirus induced broadly reactive neutralizing antibody, which may explain the observed heterologous immunity. In a study of homologous immunity, piglets were exposed to human rotavirus "Wa" in utero and again at birth. Control animals without prior exposure to rotavirus shed significantly more virus and tended to have more diarrhea than those inoculated in utero. In an additional study piglets were inoculated post-natally with rhesus rotavirus and subsequently challenged with human rotavirus ("M" strain) to look for immunity between those related strains. Such prior rotavirus infection diminished viral shedding when piglets were challenged with human rotavirus ("M" strain). Experimental animal systems now exist for the evaluation of cross-immunity between rotavirus strains, and these studies should aid in the selection of appropriate vaccine strains.

PROJECT NUMBER (Do NOT use this space)

HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00341-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

STUDIES OF ROTAVIRUSES IN VOLUNTEERS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Richard G Wyatt, M.D.	Medical Officer	LID	NIAID
	Albert Z. Kapikian, M.D.	Head, Epidemiology Section	LID	NIAID
OTHERS:	Harry Greenberg, M.D.	Medical Officer	LID	NIAID
	Robert M. Chanock	Chief	LID	NIAID

COOPERATING UNITS (if any)

Center for Vaccine Development, U. of Md. (Dr. Levine); NINCDS (Dr. William London)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
EPIDEMIOLOGY SECTION

INSTITUTE AND LOCATION
NIAID, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: 1.2	PROFESSIONAL: 0.6	OTHER: 0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Previously we observed that wild type human rotavirus (strain D contained in a suspension of feces) induced diarrheal illness in 5 of 18 adult volunteers. During this study serologic correlates of resistance were identified. Following adaptation of the Wa strain of human rotavirus to tissue culture we used this information to identify susceptible individuals so that we could investigate virulence of the Wa mutant for adult volunteers. Six of 10 volunteers with a low level of serum antibody were infected but did not become ill. This suggested that the Wa rotavirus mutant was attenuated compared to the wild type D rotavirus previously studied in adult volunteers. Three of the Wa volunteers developed a mild transaminase elevation 10 days post challenge; this response was transient and did not appear to be related to rotavirus infection. These 3 individuals again developed a mild transaminase elevation several weeks later. Chimpanzees and rhesus monkeys also developed a mild transient rise in transaminase 8 to 11 days after inoculation with the tissue culture grown Wa inoculum. The cause of the transaminase elevations is not clear at this time. Currently, 4 other rotaviruses are being evaluated for their capacity to induce transaminase elevation in primates. Those strains that do not induce this response will be considered for subsequent study in volunteers.

PROJECT NUMBER (Do NOT use this space)	HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 AI 00342-01 LID

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

STUDIES OF GASTROENTERITIS VIRUSES BY ELECTRON MICROSCOPY

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Albert Z. Kapikian, M.D.	Head, Epidemiology Section	LID NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
LABORATORY OF INFECTIOUS DISEASES

SECTION
EPIDEMIOLOGY SECTION

INSTITUTE AND LOCATION
NIAID, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The electron microscope (EM) has been a mainstay for study of fastidious gastroenteritis viruses. Although 2nd and 3rd generation tests have been developed for the detection of "Norwalk" viruses and rotaviruses, EM remains an essential tool: (1) as the "supreme court" when newer tests yield variable results, (2) in the quest for new agents of viral gastroenteritis, (3) for visualizing the site of attachment of monoclonal antibodies, (4) for serologic studies and direct visualization of particles. A fastidious 27 mm virus of epidemic gastroenteritis--the Marin County agent--was administered to volunteers in an attempt to generate particle positive stools. Particles were detected by EM in stools of one ill volunteer. The identification of the particle as the Marin County virus was established by IEM; the particle was reacted with paired sera from the original outbreak and a significant antibody rise was detected. In other studies, the site of attachment on the rotavirus particle of monoclonal antibody with subgroup specificity was studied by direct and indirect IEM. Finally, about 50% of the episodes of pediatric diarrhea are still without any known etiology. A major effort will be made to examine by IEM or indirect IEM such "negative" specimens in an attempt to detect new etiologic agents.

PHS-6040
(Rev. 10-76)

LABORATORY OF MICROBIAL IMMUNITY

1982 Annual Report
Table of Contents

Z01-A1 Project Number		Page
Summary Statement		23-1
00131-15	Mechanisms of Hypersensitivity in Inbred Histocompatible Guinea Pigs. -- Stone	23-5
00134-20	Control of Immunoglobulin Synthesis in Mice -- Asofsky	23-6
00135-08	Properties of Immunoglobulin Secreting Cells -- Morse	23-7
00136-10	Functional Activities of Subpopulations of Thymus-Derived Cells. -- Asofsky	23-8
00137-16	Biology of Graft-Versus-Host Reactions. -- Asofsky	23-9
00138-08	Viruses and the Immune Response. -- Morse	23-10
00139-17	The Immunologic Response of Animals to Trypanosomal Antigens. -- Finerty	23-11
00140-17	Immunology of Malaria. -- Finerty	23-12
00141-08	Immune Responses to Malaria and Related Intracellular Protozoa. -- Taylor	23-13
00142-09	Development of Thymus-Derived (T) Suppressor and Amplifier Cell Function. -- Baker	23-14
00143-13	Genetic Control of the Antibody Response to Type III Pneumococcal Polysaccharide (SSS-III). -- Baker	23-15
00144-18	Regulation of the Antibody Response to Pneumococcal and Other Polysaccharide Antigens. -- Baker	23-16
00145-15	Mode of Action of Thymus-Derived (T) Suppressor and Amplifier Cells. -- Baker	23-17
00146-09	Immunological Studies on Components Isolated from Bacteria, Parasites and Plants. -- Baker	23-18

Z01-A1
Project Number | Page

00153-06 *In Vitro* Response of Human Peripheral Lymphocytes to Infectious Organisms. -- Asofsky....................23-19

00158-06 The Immune Response to *Entamoeba* Antigens. -- Finerty..23-20

00186-09 Pathogenesis of Autoimmunity in Inbred Strains of Mice. .-- Chused..................................23-21

00187-09 Studies in Sjogren's Syndrome. -- Chused..............23-22

00203-03 Applications of Flow Microfluorometry -- Chused...23-23

00204-02 Immune Responses to Non-H-2 Antigens. -- Davidson...23-24

00205-02 Phenotypic and Functional Abnormalities of T and B Lymphocytes of Autoimmune Mice. -- Davidson...........23-25

PHS-NIH
SUMMARY STATEMENT

ANNUAL REPORT OF THE LABORATORY OF MICROBIAL IMMUNITY NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

October 1, 1981 to September 30, 1982

Richard Asofsky, M.D.
Chief, Laboratory of Microbial Immunity

Research in this Laboratory is concerned mainly with the differentiation of lymphoid cells and with basic mechanisms controlling cellular and humoral immune responses to microbial and tissue antigens. Blood parasites, bacterial polysaccharides, and lipopolysaccharides, as well as "integrated" leukemia viruses and histocompatibility antigens are used. Genetic studies on genes controlling lymphocyte differentiation, genes required for the expression of antigens of leukemia viruses, and genes controlling certain antibody responses are in progress. Neoplastic B-cell lymphomas are used to examine various stages of B cell differentiation. Somatic cell hybrids (hybridomas) are made to several groups of antigens. Two of the B cell lymphomas have been drug-marked, and are used to obtain B cell hybrids. Autoimmune diseases, such as the SLE-Like syndrome of NZB mice and experimental encephalomyelitis in guinea pigs are under active study.

DISCOVERY OF ANOTHER GENE THAT INFLUENCES ANTIBODY RESPONSES TO HELPER TO CELL INDEPENDENT ANTIGENS

RIII S/J mice lack an autosomal dominant gene(s) that has a decisive role in determining the capacity to make an IgM antibody to several representative helper T cell independent antigens such as Type III pneumococcal polysaccharide (SSS-III), bacterial lipopolysaccharide (LPS), ($\alpha 1 \rightarrow 3$) dextran, and bacterial levan. These mice appear to possess functionally active helper T cells since they give a reasonably good antibody response to sheep erythrocytes, a helper T cell-dependent antigen. The genetic defect is not due to the absence of a subset of B cells capable of giving a mitogenic response to LPS. Although the genetic defect of RIII S/J mice resembles the defect found in CBA/N mice it is not X-linked. Further, studies on the nature of this genetic defect may provide important new information on genetic factors involved in antigen-induced B cell activation. (P.J. Baker, J. Hiernaux, F. M. Rollwagen, T. Chused, P. W. Stashak, and D. F. Amsbaugh, LMI, NIAID; J. A. Rudbach, University of Montana; and B. Prescott, Biomedical Research Institute, Rockville, MD).

DEVELOPMENT OF IMMUNOLOGICAL MEMORY PROCEEDS IN A CYCLIC MANNER

The antibody response of mice to *Escherichia coli* 0113 lipopolysaccharide (LPS) displays a cyclic (oscillatory) pattern that is T cell dependent and does not involve the formation of auto-anti-idiotypic antibody. Since the development of immunological memory to LPS is also T cell dependent, we examined the role of immunological memory in the expression of the cyclic pattern observed. The results of these studies showed that memory likewise develops in a cyclic manner and is likely to play a major role in modulating the magnitude of the antibody response to this antigen. (P.J. Baker, J. Hiernaux, P. W. Stashak, and J. Chiang, LMI, NIAID; C. Delisi, LTB, NIC; and J. A. Rudbach, University of Montana).

ANTIBODY RESPONSES TO P. YOELII IN MICE ARE ALL T DEPENDENT

Based on results from previous studies, it is unclear if rodent malarial parasites possess T-independent (TI) antigens (AGS), and if they do, what the role of TI Ags might be in immunity: Thus, we studied TI responses to *Plasmodium yoelii in vivo* and *in vitro*. AB to *P. yoelii* could not be demonstrated in the plasma of athymic (nu/nu) mice infected with *P. yoelii*. However, hybridomas secreting antimalaria Abs could be produced using spleen cells form athymic mice. This suggests that athymic mice have Ag-responsive B cells but Abs were not produced due to lack of T cell help. Thus, *P. yoelii* parasites lack conventionally defined type 2 TI Ags. In addition, purified B cells from normal mice, which were responsive to LPS, did not proliferate or secrete Ig *in vitro* in response to malarial extracts. These results suggest that *P. yoelii* lacks both type 1 and 2 TI Ags. (Drs. D. W. Taylor, C. Bever, F. M. Rollwagen, R. Asofsky, and Mr. C. B. Evans, LMI, NIAID).

STUDIES OF SJL MICE AND MICE CARRYING ECOTROPIC VIRUS LOCI

NFS mice congenic for ecotropic MuLV *V* loci have been bred by Dr. W.P.Rowe. These mice develop a high incidence of non-thymic lymphomas which histologically resemble reticulum cell sarcoma Type B as described by Dunn. This type of tumor is characteristic of the disease exhibited by SJL mice 8-18 months of age, some of which can be transplanted. Tumors from NFS *V* congenic mice and SJL transplantable tumors were tested to determine their relationship to normal T and B cell lineages. SJL tumors were found to have cell surface markers characteristic of early B cells and NFS tumors had markers which define cells but early and later in the B cell lineage. Some of the NFS tumors have been cultured *in vitro* and cloned and maintain their B cell characteristics.

GENE CONTROLLING EXPRESSION OF XENOTROPIC LEUKEMIA VIRUSES (Cxv-1) MAPPED TO THE H-2 COMPLEX OF MICE

A new locus (Cxv-1) governing expression of infectious xenotropic murine leukemia viruses (MuLV) has been mapped to the I-C region of the H-2 complex. Strains F/St, B10.F, and B10.P, all of the $H\text{-}2^p$ haplotype, carry an allele at this locus which is premissive for high expression of xenotropic viruses for early in life. Strains with H-2 haplotypes k, d, b,and s carry a restrictive allele that is dominant to the permissive allele. This locus controls the extent to which virus is produced for chromosome 1 loci for xenotropic MuLV. Deregulation of virus expression if associated with development of early tumors in B10.F and B10.P mice. These results indicate that the major histocompatibility complex may affect tumor development through control of endogenous virus expression. (Drs. Morse, Yetter and Davidson, LMI, and Drs. Hartley and Kozak, LVD).

B CELL HYBRIDOMAS STIMULATED TO SECRETE IMMUNOGLOBULIN

Somatic cell fusion *in vitro* between a B cell lymphoma from BALB/c mice and normal B cells of C57BL/6 (B6) mice has produced a group of stable B cell hybrids which express mIgM of B6 origin and Ia and H-2 antigens of both B6 and BALB/c origin. Stimulation of these hybridoma lines *in vitro* results in secretion of sIgM by 20-30% of cells. Secretion follows a slowing of cell division. T cell factors and bacterial lipopolysaccharide are less efficient stimulations of this differentiation. One hybridoma line secretes IgG2a after stimulation. (Drs. Hamano and Asofsky).

ADMINISTRATIVE

Dr. Alain Paraf, a Guest Worker from France on sabbatical leave, returned to Paris. Dr. Christopher Bever completed a 2-year research associateship, and joined NINCDS.

Dr. Carol Sulis joined the Laboratory as a Medical Staff Fellow in July, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-A1-00131-15-LMI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mechanisms of Hypersensitivity in Inbred Histocompatible Guinea Pigs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. H. Stone	Head, Experimental Autoimmunity Section	LMI/NIAID
Other:	D. F. Amsbaugh		LMI/NIAID
	R. B. Nussenblatt	Senior Investigator	CB/NEI
	M. B. Datiles		CB/NEI
	Tian-Sheng Hu		LVR/NEI
	C. S. Raine*		
	U. Traugott*		

COOPERATING UNITS (if any)

*Division of Neuropathology, Albert Einstein College of Medicine
New York City

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Experimental Autoimmunity Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have been studying allergic encephalomyelitis (EAE) in juvenile guinea pigs which undergo a chronic stage after an acute attack or silent acute stage characterized by histological changes in the CNS without clinical signs. Using monoclonal antibodies against guinea pig T cells and antibodies against immunoglobulins, T and B cells were traced to their CNS sites. In acute EAE, T cells appeared before B cells and were distributed within the white matter parenchyma while B cells remained in perivascular spaces. In chronic EAE which is reminiscent of multiple sclerosis in man, T cells were also localized within the parenchyma. B cell infiltrates were more extensive than in acute EAE, and although most were centered around blood vessels, some were in the parenchyma. In chronic EAE treated with myelin basic protein (MBP), disease was arrested, and T and B infiltrates in the white matter negligible. In other studies on treatment of chronic EAE, marked clinical improvement was noted when a lipid hapten of myelin, galactocerebroside (GC) was added to the MBP. The rationale stemmed from work suggesting that MBP was responsible for sensitization of T cells and GC was important in producing demyelinating antibodies, and possibly both were important in the production of disease.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-A1-00134-20-LMI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Control of Immunolobulin Synthesis in Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Asofsky	Laboratory Chief	LMI/NIAID
Other:	K. J. Kim	Visiting Associate	LMI/NIAID
	T. Hamano	Visiting Fellow	LMI/NIAID
	C. Bever	Research Associate	LMI/NIAID
	A. Paraf	Guest Worker	LMI/NIAID
	F. Rollwagen	Staff Fellow	LMI/NIAID
	B. J. Fowlkes	Microbiologist	LMI/NIAID
	A. Brooks	Bio Lab Tech	LMI/NIAID
	C. Evans	Bio Lab Tech	LMI/NIAID
	W. Leiserson	Mathematician	LMI/NIAID
	S. O. Sharrow	Chemist	LMI/NIAID

COOPERATING UNITS (if any)
R. Laskov, Hadassah Medical Center, Jerusalem; G. J. Thorbecke, I. Katz, NYU Medical Center; G. Siskind, E. Goidl, Cornell Medical Center; A. Penn, Hai-Won Chung, P. and S., Columbia University

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Experimental Pathology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	4.2	1.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

M12.4.1, an m IgM B cell lymphoma of strain BALB/c mice was drug marked, and B cell hybridomas obtained by somatic cell fusion of this cell line with normal B-lymphocytes from strains C57BL/6J, A/J and the F_1 (BALB/c x A/J). All such hybridomas bear H-2 and Ia antigens from the normal cells on their membranes; all express m IgM. The hybridomas could be induced to secrete IgM, and in one case IgG_{2a}, by treatment in vitro with anti IgM. Lipopolysaccharide from E. coli, ~~and T~~ cell factors generated in vitro were less efficient inducers.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-A1-00135-08-LMI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Properties of Immunoglobulin Secreting Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Herbert C. Morse, III   Senior Investigator   LMI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Virology and Cellular Immunology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS   ☐ (b) HUMAN TISSUES   ☒ (c) NEITHER

☐ (a1) MINORS   ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Inactive during current year.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AI-00136-10-LMI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Functional Activities of Subpopulations of Thymus-Derived Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Asofsky	Laboratory Chief	LMI/NIAID
	B. J. Mathieson	Member, Basel Institute for Immunology	
Other:	B. J. Fowlkes	Microbiologist	LMI/NIAID
	A. Brooks	Medical Tech	LMI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Experimental Pathology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.1	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Velocity sedimentation at unit gravity was used to separate bone marrow cells. Precursors of hematopoeitic cells, as judged both by a colony forming assay, and by staining with a monoclonal antibody specific for such cells, were separated completely from cells bearing Ly 9, a cell surface antigen of lymphoid cells. Many of the cells positive for Ly 9 lacked markers of either B lymphocytes or T lymphocytes. The presence of Lyt-2, a surface antigen of murine thymocytes and of T lymphocytes was highly correlated among thymocytes from adult mice with bright staining with fluorochrome labeled PNA (peanut lectin), e.g. there were 2 populations: PNA bright, Lyt 2+ and PNA dull, Lyt 2-. In contrast, four populations are found in fetal thymus; Lyt 2+ and Lyt 2- in the both the PNA bright and dull populations. Fluorochrome labeled lectins appear to show subpopulations of thymocytes and other T lymphocytes as judged by degree of staining measured in a fluorescence activated cell sorter. Cell sorting or lectin-affinity cell separation methods should permit separation of these cell population for functional study.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 A1 00137-16 LMI

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Biology of Graft-Versus-Host Reactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Richard Asofsky	Laboratory Chief	LMI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Experimental Pathology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Inactive during current year.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-A1-00138-08-LMI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Viruses and the Immune Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Herbert C. Morse, III	Head, Virology & Cellular Immunology Section	LMI/NIAID
Other:	J. W. Hartley	Senior Investigator	LVD/NIAID
	R. A Yetter	Guest Researcher (IPA)	LMI/NIAID
	C. A. Kozak	Staff Fellow	LVD/NIAID
	W. F. Davidson	Expert/Consultant	LMI/NIAID
	W. Y. Langdon	Fogarty Fellow	LMI/NIAID
	E. Rudikoff	Bio Lab Tech	LMI/NIAID
	T. N. Fredrickson	Guest Researcher (IPA)	LVD/NIAID
	J. Ihle	Senior Investigator	FCRC/NCI
	S. Ruscetti	Staff Fellow	VCP/NCI
	J. Selver, C. Buckler		LVD/NIAID
	P. M. Hoffman*, B. A. Taylor**, E. Stickert***, J. H. Stimplfing****		

COOPERATING UNITS (if any)
*University of South Carolina, Charleston, South Carolina
**The Jackson Laboratory, Bar Harbor, Maine
***Memorial Sloan Kettering, New York, New York

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Virology and Cellular Immunology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A major gene controlling expression of endogenous xenotropic murine leukemia viruses (x-MuLV) was mapped to the I-C region of the H-2 complex. The gene, termed Cxv-1, has an allele permissive for high levels of virus expression in mice bearing the $H\text{-}2^p$ haplotype whereas most other strains carry a restrictive allele at this locus. The gene appears to determine the levels of expression of infectious virus from virus-inducing loci on chromosome 1.

An extract from the spleen of a lymphomatous mice infected with the wild mouse ecotropic MuLV, CAS-BR-M, induced erythroleukemias in newborn mice after a latent period of 4 to 12 weeks. The extracts were found to contain a defective agent similar to but distinct from the spleen focus forming virus of the Friend MuLV complex. Other extracts which do not contain defective MuLV were found to rapidly induce lymphomas.

NFS mice, congenic for ecotropic MuLV loci, and SJL mice develop splenic lymphomas previously characterized as reticulum cell sarcoma, Type B. Tumors from both sets of mice were shown to bear cell surface antigens characteristic of cells in the B lymphocyte lineage.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-A1-00139-17-LMI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Immunologic Response of Animals to Trypanosomal Antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. F. Finerty	Research Microbiologist	LMI/NIAID
Other:	D. M. Dwyer	Research Microbiologist	LPD/NIAID
	P. Gardiner	Visiting Fellow	LPD/NIAID
	C. T. Hansen	Geneticist	DRS
	L. Kendrick	Biologist	LMI/NIAID
	L. P. Gasbarre*	Research Microbiologist	
	J. Jones**	Assistant Professor	

COOPERATING UNITS (if any)

*Laboratory of Parasitology, USDA, Beltsville, MD
**Department of Microbiology, Jefferson Medical School, Philadelphia, PA

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Microbiology and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mice infected with African trypanosomes serve as a model of the human disease. Assays of specifi Ig isotype responses indicate a class restricted Ig response response to a glycoprotein antigen. Infection of nude mice induces T cell maturation within 14-18 days. A new immunodiagnostic method was developed for assaying stage specific response.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-A1-00138-08-LMI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Viruses and the Immune Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Herbert C. Morse, III	Head, Virology & Cellular Immunology Section	LMI/NIAID
Other:	J. W. Hartley	Senior Investigator	LVD/NIAID
	R. A Yetter	Guest Researcher (IPA)	LMI/NIAID
	C. A. Kozak	Staff Fellow	LVD/NIAID
	W. F. Davidson	Expert/Consultant	LMI/NIAID
	W. Y. Langdon	Fogarty Fellow	LMI/NIAID
	E. Rudikoff	Bio Lab Tech	LMI/NIAID
	T. N. Fredrickson	Guest Researcher (IPA)	LVD/NIAID
	J. Ihle	Senior Investigator	FCRC/NCI
	S. Ruscetti	Staff Fellow	VCP/NCI
	J. Selver, C. Buckler		LVD/NIAID
	P. M. Hoffman*, B. A. Taylor**, E. Stickert***, J. H. Stimplfing****		

COOPERATING UNITS (if any)
*University of South Carolina, Charleston, South Carolina
**The Jackson Laboratory, Bar Harbor, Maine
***Memorial Sloan Kettering, New York, New York

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Virology and Cellular Immunology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A major gene controlling expression of endogenous xenotropic murine leukemia viruses (x-MuLV) was mapped to the I-C region of the H-2 complex. The gene, termed Cxv-1, has an allele permissive for high levels of virus expression in mice bearing the $H\text{-}2^p$ haplotype whereas most other strains carry a restrictive allele at this locus. The gene appears to determine the levels of expression of infectious virus from virus-inducing loci on chromosome 1.

An extract from the spleen of a lymphomatous mice infected with the wild mouse ecotropic MuLV, CAS-BR-M, induced erythroleukemias in newborn mice after a latent period of 4 to 12 weeks. The extracts were found to contain a defective agent similar to but distinct from the spleen focus forming virus of the Friend MuLV complex. Other extracts which do not contain defective MuLV were found to rapidly induce lymphomas.

NFS mice, congenic for ecotropic MuLV loci, and SJL mice develop splenic lymphomas previously characterized as reticulum cell sarcoma, Type B. Tumors from both sets of mice were shown to bear cell surface antigens characteristic of cells in the B lymphocyte lineage.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-A1-00139-17-LMI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Immunologic Response of Animals to Trypanosomal Antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. F. Finerty	Research Microbiologist	LMI/NIAID
Other:	D. M. Dwyer	Research Microbiologist	LPD/NIAID
	P. Gardiner	Visiting Fellow	LPD/NIAID
	C. T. Hansen	Geneticist	DRS
	L. Kendrick	Biologist	LMI/NIAID
	L. P. Gasbarre*	Research Microbiologist	
	J. Jones**	Assistant Professor	

COOPERATING UNITS (if any)

*Laboratory of Parasitology, USDA, Beltsville, MD
**Department of Microbiology, Jefferson Medical School, Philadelphia, PA

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Microbiology and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mice infected with African trypanosomes serve as a model of the human disease. Assays of specifi Ig isotype responses indicate a class restricted Ig response response to a glycoprotein antigen. Infection of nude mice induces T cell maturation within 14-18 days. A new immunodiagnostic method was developed for assaying stage specific response.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-A1-00140-17-LMI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)
Immunology of Malaria

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. F. Finerty	Research Microbiologist	LMI/NIAID
Other:	L. Kendrick	Biologist	LMI/NIAID
	J. Langhorne	Visiting Fellow	LMI/NIAID
	W. P. Weidanz*	Professor	
	F. Rollwagen	Staff Fellow	LMI/NIAID

COOPERATING UNITS (if any)
*The Hahnemann Medical College, Philadelphia, PA

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Microbiology and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies on the effect of non-lethal malarial infections on lethal malaria infections indicate early immune responses can be altered to protect the host. A new method was developed to assay stage specific host responses to malarial parasites. An in vitro assay to measure specific antibodies to Plasmodium chabaudi was developed. The assay will determine specific T cell help and suppression during the course of a malarial infection.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-A1-00141-08-LMI

PERIOD COVERED

October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immune Responses to Marlaria and Related Intracellular Protozoa

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. W. Taylor	Senior Investigator	LMI/NIAID
	R. Asofsky	Laboratory Chief	LMI/NIAID
Other:	J. Langhorne	Visiting Associate	LMI/NIAID
	F. M. Rollwagen	Staff Fellow	LMI/NIAID
	C. T. Bever	Research Associate	LMI/NIAID
	C. B. Evans	Biological Lab Tech	LMI/NIAID
	E. Pacheco	MBS Summer Student	LMI/NIAID
	J. F. Rowe	MBS Summer Student	LMI/NIAID
	S. L. Henderson	MBS Student	LMI/NIAID
	K. L. Middleton	Summer Student	LMI/NIAID
	J. K. Frenkel*		

COOPERATING UNITS (if any)

*J. K. Frenkel - University of Kansas

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Microbiology and Immunology Section/Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.9	2.1	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies characterizing malarial antigens and the humoral response of mice to Plasmodium yoelii were continued. During this year we produced, cloned, and cryopreserved 137 anti-P. yoelii hybridoma cell lines. These can now be used to identify biologically important antigens. In addition, a series of experiments were undertaken to determine 1) the number and molecular size of malarial immunogens that stimulate Ab production during malaria, 2) the requirement for T cells in antimalaria Ab production, and 3) the kinetics of Ab induction by non-species specific (crossreactive) Plasmodial Ags. Results from these studies demonstrate that P. yoelii parasites have at least 33 protein immunogens that the Ags require T cells for Ab production, i.e. P. yoelii lacks T-independent Ags, and that antigenic determinants (i.e. epitopes) common to related malaria parasites may be present on immunogens of different molecular weights. Experiments were also initiated this year to determine if a correlation exists between the kinetics of Ab response to stimulatory malarial Ags and the genetics of host resistance to rodent malaria.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-A1-00142-09-LMI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Development of Thymus-Derived (T) Suppressor and Amplifier Cell Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.J. Baker	Head, Microbiology & Immunology Section	LMI/NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Microbiology and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.3	PROFESSIONAL: 0.5	OTHER: 0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Inactive during current year.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-A1-00143-13-LMI

PERIOD COVERED

October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Genetic Control of the Antibody Response to Type III Pneumococcal Polysaccharide (SSS-III)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.J. Baker	Head, Microbiology & Immunology Section	LMI/NIAID
	J. Hiernaux	Visiting Fellow	LMI/NIAID
Other:	P.W. Stashak	Microbiologist	LMI/NIAID
	D.F. Amsbaugh	Biologist	LMI/NIAID
	G. Caldes	Chemist	LMI/NIAID
	F.M. Rollwagen	Staff Fellow	LMI/NIAID
	T. Chused	Senior Investigator	LMI/NIAID
	B. Prescott*		
	J.A. Rudbach**		

COOPERATING UNITS (if any)

*Biomedical Research Institute, Rockville, MD 20205
**University of Montana, Missoula, Montana

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Microbiology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

RIII S/J mice lack an autosomal dominant gene(s) that has a decisive influence in determining the capacity to make an IgM antibody response to several representative helper T cell-independen antigens; these mice appear to possess functionally active helper T cells since they make a reasonably good antibody response to sheep erythrocytes, a helper T cell dependent antigen. Studies on the genetic defect involved, which cannot be attributed to the absence of B cells capable of giving a mitogenic response to bacterial lipopolysaccharide (LPS), may provide important new information on the genetic factors involved in antigen-induced B cell activation.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AI-00144-18-LMI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Regulation of the Antibody Response to Pneumococcal and Other Polysaccharide Antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.J. Baker	Head, Microbiology & Immunology Section	LMI/NIAID
	J. Hiernaux	Visiting Fellow	LMI/NIAID
	P.W. Stashak	Microbiologist	LMI/NIAID
	J. Chiang	Summer Student	LMI/NIAID
Other:	B. Prescott*		
	C. DeLisi**		
	J.A. Rudbach***		

COOPERATING UNITS (if any)
*Biomedical Research Institute, Rockville, Md 20205
**Laboratory of Theoretical Biology, NCI, Bethesda, MD 20205
***University of Montana, Missoula, Montana

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Microbiology and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.4	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The antibody response of mice to an optimally immunogenic dose of Escherichia coli 0113 lipopolysaccharide (LPS) displays a cyclic (uscillatory) pattern. Although the development of this cyclic pattern is T cell dependent, it does not appear to involve the formation of auto - anti-idiotypic antibody. In studies designed to identify those factors that contribute to the expression of the cyclic pattern, we observed that the development of immunological memory to LPS also proceeds in a cyclic manner. Since the development of memory is T cell dependent, it appears to play a major role in modulating the magnitude of the antibody response to this antigen.

In contrast to the antibody response to LPS, the antibody response to Type III pneumococcal polysaccharide (SSS-III) does not proceed in a cyclic manner; also, it is not possible to demonstrate immunological memory to this antigen. Nevertheless, the expression of suppressor T cell activity, which plays an important role in regulating the magnitude of the antibody response to SSS-III, is expressed in a cyclic manner. Such cycling is due mainly to changes in the rate of proliferation of activated suppressor T cells.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-A1-00145-15-LMI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mode of Action of Thymus-derived (T) Suppressor and Amplifier Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.J. Baker	Head, Microbiology & Immunology Section	LMI/NIAID
Other:	C.E. Taylor	Visiting Fellow	LMI/NIAID
	P.W. Stashak	Microbiologist	LMI/NIAID
	D.F. Amsbaugh	Biologist	LMI/NIAID
	G. Caldes	Chemist	LMI/NIAID
	M. Fauntleroy	Co-op Student	LMI/NIAID
	B. Prescott*		

COOPERATING UNITS (if any)

*Biomedical Research Institute, Rockville, MD 20205

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Microbiology and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.1	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Prior treatment (priming) with a subimmunogenic dose of Type III pneumococcal polysaccharide (SSS-III) results in an antigen-specific T-cell dependent form of unresponsiveness (low-dose paralysis). Such unresponsiveness can be transferred by spleen cells obtained 5-24 hr after priming. The suppressive activity of transferred cells is abolished by prior treatment with monoclonal anti-Thy 1, anti-Lyt-2, and anti-I-J antibody in the present of complement; thus, suppression is mediated by a distinct subset of T cells (suppressor T cells). However, if primed spleen cells are transferred 24-72 hrs after immunization with SSS-III, the resulting antibody response of immunized recipients is enhanced, rather them suppressed. Greater enhancement is noted using transferred cells pretreated with monoclonal anti-Lyt2 antibody plus complement to remove suppressor T cells; such enhancement is attributed to amplifier T cells having the Lyt-1 phenotype. These findings indicate that suppressor T cells regulate the antibody response to SSS-III by influencing the expansion of SSS-III - specific clones of B cells as well as the expression of amplifier T cell activity; the latter cause B cells to proliferate further in response to SSS-III.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-A1-00146-09-LMI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immunological Studies of Components Isolated from Bacteria, Parasites and Plants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Caldes	Chemist	LMI/NIAID
Other:	P.J. Baker	Head, Microbiology and Immunology Section	LMI/NIAID
	B. Prescott*		
	P.W. Stashak	Microbiologist	LMI/NIAID
	D.F. Amsbaugh	Biologist	LMI/NIAID

COOPERATING UNITS (if any)

*Biomedical Research Institute, Rockville, MD 20205

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Microbiology and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.1	OTHER: 0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A commerically available pectinase from Aspergillus niger was found to be effective in hydrolyzing, to their constitnent monosaccharides, polysaccharides from Carthamus tinctorious flowers, Type III pneumococcal polysaccharide and other nonpectic saccharides.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 A1 00153-06 LMI

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

In vitro Response of Human Peripheral Lymphocytes to Infectious Organisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Richard Asofsky	Laboratory Chief	LMI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

No progress was made in the past year.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-A1-00158-06-LMI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Immune Response to Entamoeba Antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. F. Finerty	Research Microbiologist	LMI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Microbiology and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Inactive during current year.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-A1-00186-09-LMI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Pathogenesis of Autoimmunity in Inbred Strains of Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. M. Chused	Senior Investigator	LMI/NIAID
Other:	V. Manohar	Visiting Fellow	LMI/NIAID
	H. A. Wilson	Guest Worker	LMI/NIAID
	E. Brown	Biologist	LMI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Virology and Cellular Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The immunologic mechanism and genetic control of autoimmune disease is being investigated in autoimmune mice utilizing flow microfluorometry and functional analysis of lymphocyte subpopulations.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-A1-00187-09-LMI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)
Studies in Sjogren's Syndrome

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. M. Chused	Senior Investigator	LMI/NIAID
Other:	E. M. Brown	Biologist	LMI/NIAID
	H. Moutsopoulos	Visiting Investigator	LMI/NIDR
	T. Lawley	Senior Investigator	D/NCI
	D. Mann	Senior Investigator	I/NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Virology and Cellular Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Inactive during current year.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-A1-00203-03-LMI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Applications of Flow Microfluorometry

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. M. Chused	Senior Investigator	LMI/NIAID
Other:	W. M. Leiserson	Mathematician	LMI/NIAID
	V. Manohar	Visiting Fellow	LMI/NIAID
	H. A. Wilson	Guest Worker	LMI/NIAID
	E. Brown	Biologist	LMI/NIAID

B. E. Seligmann, Research Associate, I. Gallin, Senior Investigator, M. Berger, Research Associate, M. Frank, Clinical Director, Chiam Brickman, Clinical Associate, Eric Brown, Clinical Associate, LCI/NIAID; G. Weil, Research Associate, LPD/NIAID; I. Levy, Computer Specialist, DCRT/NIH, M. Wilkinson, Visiting Fellow, Sue Jackson, Tom Kindt, Chief, LIG/NIAID; J. Smollen, Visiting Fellow, A. F. Steinberg, Senior Investigator, ARB/NIAMDD; R. Hall, Senior Investigator, T. Lawley, Senior Investigator, T. Storch, Research Associate, Stuart Aronson, Chief, DB/NCI.

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Virology and Cellular Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	0.9	1.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Flow microfluorometry, supported by multiparamater data analysis is being applied to problems in multiple areas: 1) regulation of membrane potential by lymphocytes and neutrophils, 2) lymphocyte ontogeny and activation, 3) abnormalities in lymphocyte subpopulations associated with murine autoimmune disease, and 4) lymphocyte and monocyte analysis in human immunologic diseases.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-A1-00204-02-LMI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immune Responses to Non-H-2 Antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Wendy F. Davidson	Expert/Consultant	LMI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Virology and Cellular Immunology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Project to be terminated.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-A1-00205-02-LMI

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Phenotypic and Functional Abnormalities of T and B Lymphocytes of Autoimmune Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Wendy F. Davidson	Expert/Consultant	LMI/NIAID
	H. C. Morse III	Senior Investigator	LMI/NIAID
Other:	T. M. Chused	Senior Investigator	LMI/NIAID
	C. Kozak	Staff Fellow	LVD/NIAID
	B. J. Mathieson	Senior Staff Fellow	LMI/NIAID
	J. Roths*		
	E. Murphy*		
	R. Coffman**		
	L. Schultz*		

COOPERATING UNITS (if any)
*The Jackson Laboratory, Bar Harbor, Maine
**Stanford University, Palo Alto, Calif.

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Virology and Cellular Immunology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Research has involved the study of the immunologic and genetic mechanisms involved in the development and expression of autoimmune disease. Four murine models of autoimmune disease; NZB and PN mice and mice homozygous for the mutant genes lpr or me were studied for phenotypic and functional abnormalities of T and B lymphocytes prior to and after the onset of severe disease. B cells from each autoimmune strain were hyperactive as evidenced by a decrease in the level of expression of cell surface IgM and IgD, a marked increase in the frequency of splenic plasma cells and cells spontaneously secreting immunoglobulin and the production of autoantibodies. In contrast to the other strains, lpr/lpr mice had a high incidence of Thy 1$^+$, Ly 1$^+$, 2$^-$ T cells that also bore markers normally only expressed on B lymphocytes. Gene reorganization studies showed an rearrangement of immunoglobulin heavy chain genes in these cells. This abnormal T cell population was deficient in T helper cell activity and produced decreased amounts of Interleukin-2. B6 me/me mice also had unique abnormalities of T cells. These included a decrease in the frequency of all T cells in the spleen and an increase in the proportion of Ly2$^+$ T cells in lymph node. Severe abnormalities in T lymphocyte function were in evidence as early as 4 weeks of age.

PHS-NIH
SUMMARY REPORT

ANNUAL REPORT OF THE LABORATORY OF MOLECULAR MICROBIOLOGY, NIAID

October 1, 1981 - September 30, 1982

Dr. Malcolm A. Martin
Chief, Laboratory of Molecular Microbiology

During its first full year of operation, the Laboratory of Molecular Microbiology (LMM) added a new section and began the consolidation of research activities involving RNA and DNA tumor viruses, tumor immunology, streptococcal plasmids, and mycoplasmas. Functioning more like a microbiology department with a heavy emphasis on molecular biological approaches, the LMM at age 16 months is administratively divided into four programmatic sections, two located in Building 5 on the Bethesda campus and two situated in Building 550 of the Frederick Cancer Research Facility, Frederick, Maryland.

The Biochemical Virology Section (Dr. Malcolm Martin, Head) conducts research involving RNA and DNA tumor viruses focussing attention on the molecular structure, integration, and expression of viral genomes during lytic and transforming infection. The Viral Biology Section (Dr. Kenneth Takemoto, Head) is concerned with the identification and biological characterization of new virus isolates and studies viral and cellular factors associated with oncogenic transformation and tumorigenesis. The Bacterial Virulence Section (Dr. Donald LeBlanc, Acting Head) carries out a program involving plasmid biology concentrating mainly on the plasmids of streptococci. The Mycoplasma Section (Dr. Joseph Tully, Head) characterizes and classifies mycoplasmas isolated from man and other animals.

Following the disestablishment of the Laboratory of Streptococcal Diseases, two members of its scientific staff (Drs. Theodore and Ranhand) and two members of its technical staff (Mr. Willard Davis and Ms. Blanche Lewis) were administratively transferred to LMM. Dr. Theodore was assigned to the Biochemical Virology Section where, after learning many of the rudiments of molecular biological procedures (restriction enzyme digestions, and gel electrophoresis, nucleic acid hybridization, cloning in plasmid and lambda phage systems), he is attempting to clone size-fractionated segments of mammalian DNA using cosmid-vector systems. Dr. Ranhand was transferred to the Bacterial Virulence Section and physically moved his laboratory from Building 7 to the LMM laboratory at FCRF. As his studies involving bacterial competence wind down, Dr. Ranhand is expected to pursue a research program focussing on the structure and function of streptococcal plasmids which will be conducted in close collaboration with Dr. LeBlanc. Mr. Davis and Ms. Lewis have been assigned to support the research activities of Drs. Khan and Takemoto, respectively. In January, 1982, Dr. Joseph Tully, Mr. Colis Blood, and Mr. David Rose were transferred from the Laboratory of Infectious Diseases to LMM and moved to Building 550, FCRF in the spring of 1982 constituting the Mycoplasma Section of LMM.

A serious attempt has been made to integrate the varied scientific activities conducted in the LMM and encourage intellectual cross-fertilization among its scientific staff. During the past year, weekly staff meetings were held at which time investigators informally presented progress reports of their research programs. Considerable time was spent introducing fundamentals of virology, bacteriology, molecular cloning, and tumor immunology. Following the renovation of laboratory space in Building 550, the LMM held one of its staff conferences in Frederick. It is anticipated that one meeting per month will be held at the FCRF during the next year.

Considerable progress has been made in the various programmatic areas, particularly in studies elucidating the structure of integrated viral genomes and the molecular characterization of streptococcal plasmids. The strength of LMM, which is in the areas of recombinant DNA technology, biochemical virology, and molecular genetics, has been complemented by the extremely productive collaborations with members of the Laboratory of Viral Diseases who have provided a wealth of expertise in the area of viral and cellular biology which has increased the breadth of our scientific programs.

Research Progress

Cloning and characterization of endogenous MuLV-related sequences from AKR and BALB/c mice. Twelve different MuLV-related clones were obtained from a BALB/c embryo library or by shotgun cloning of size-fractionated AKR mouse DNA. All of the clones contained long terminal repeats (LTRs) as well as other genes associated with MuLVs such as reverse transcriptase, gag, and the glycoprotein envelope. Nucleotide sequencing of endogenous LTR segments indicated the presence of a novel 110 bp insert which is not found in the LTR's infectious ecotropic and xenotropic MuLVs. Other LTR regulatory sequences such as the TAATAA box and the polyadenylation addition signal were present in the endogenous clones. Experiments to evaluate the functional role of endogenous LTRs are currently in progress. (Khan and Martin)

Cloning of MuLV-related sequences from human DNA. We have obtained and extensively characterized 31 different MuLV-related clones from human DNA. A majority of the human endogenous retroviral DNA segments resemble mammalian proviruses in structure containing LTR, gag, and reverse transcriptase regions but also have a deletion of envelope (env) glycoprotein sequences. Approximately 20% of the cloned MuLV-like segments seem to contain an intact env region and may represent complete proviruses. The functional activity of the LTRs associated with human retroviral DNA segments is being evaluated using an in vitro assay in which they replace the SV40 72 bp direct repeat and activate a linked chloramphenicol acetyl transferase gene. (Steele, Rabson, and Martin)

Sequencing of murine leukemia virus and human retroviral DNA segments. The sequence of approximately 2600 nucleotides of an MuLV-related human retroviral clone was determined and shown to resemble the p12, p30, and p10 gag coding regions and the reverse transcriptase genes of Moloney leukemia virus. This sequencing analysis permitted the precise alignment of most of the cloned human DNAs with prototype MuLV genomes and indicated extensive conservation of reverse transcriptase genes.

In other studies, the nucleotide sequence of the envelope (env) regions of ecotropic and xenotropic MuLVs was determined. The two env specific sequences which determine the host range of the murine retrovirus shared short stretches of homology but contained regions of insertions/deletions with respect to one another which presumably determine the tissue tropism of each virus. (Repaske and Martin)

Differential subcellular localization of phosphorylated and non-phosphorylated middle-sized T antigens of polyoma virus and the relationship of this location to middle-sized T antigen phosphorylating activity in vitro. A small fraction of the polyoma virus middle-sized T antigen located on the plasma membrane contains an associated tyrosine-specific protein kinase activity (MT kinase) which is monitored using an *in vitro* assay. A naturally phosphorylated form of polyoma middle-sized T antigen has also been identified in the plasma membrane; up to 50% of non-phosphorylated middle-sized T antigen is present in the nuclear fraction. Results of phosphoamino acid analysis and phosphatase treatment suggest that the active form of MT kinase is phosphorylated (*in vivo*) at a serine residue and specifically adds phosphate to tyrosine residues in substrate polypeptides (Ito and Segawa).

Antiserum against a synthetic polypeptide representing the unique region of middle-sized T antigen blocks the protein kinase activity. Antiserum was raised in rabbits against a synthetic polypeptide Glu-Glu-Glu-Glu-Tyr-Met-Pro-Met-Glu, which represents the portion of the middle-sized T antigen containing the tyrosine residue which is phosphorylated in the MT kinase reaction. This antiserum reacts with SDS-denatured as well as native middle-sized T antigen. The only major protein immunoprecipitated from the extract of infected cells with the antiserum is middle-sized T antigen and this immunoprecipitation is blocked by the addition of the antibody to the nonapeptide to the extract. Immunoprecipitates obtained with the nonapeptide antiserum do not possess demonstrable MT kinase activity, indicating that the antibody covers the phosphate acceptor (tyrosine) used in the *in vitro* MT kinase reaction (Ito and Segawa).

Isolation and characterization of polyoma virus mutants capable of growing in embryonal carcinoma and trophoblast cells. Polyoma virus mutants capable of growing in the embryonal carcinoma (EC) cell line, F9, and trophoblast cell lines have been isolated. The mutants isolated from both types of cells contain a mutation in the viral regulatory region located near the origin of DNA replication. Mutants isolated from F9 cells grow in both F9 and trophoblast cells while mutants isolated from trophoblast cells replicate in trophoblast cells but not in F9 cells. Using several different cell lines, it was determined that mutants were able to replicate in more highly differentiated cells than those from which they were originally isolated and were unable to grow in less differentiated cell lines. Characteristic nucleotide sequence changes were identified in EC and trophoblast mutants of polyoma virus using the Maxam-Gilbert sequencing technique (Ito and Segawa).

Characterization of chromosomal sites of Py viral DNA integration in tumor cell DNA. The DNA sequence of seven host-viral junctions has been determined and found to contain a stem-core structure consisting of both viral and cellular sequences, 4-8 bp direct repeats in chromosomal and neighboring viral DNA sequences, and short stretches of partial homology between viral and cellular DNA located within the host cell chromosome approximately 50-100 bp away from the site of viral DNA integration. (Chowdhury and Israel)

Evaluation of polyoma pathogenicity and tissue tropism. In conjunction with Dr. Clyde Dawe (NCI) we have initiated studies to characterize genomic differences in wild-type Py strains of varying virulence. Current data suggest that virulence may be determined by a non-coding region of the genome near the origin of replication which seems to modulate the ability of various viruses to replicate in mouse kidneys. (Chowdhury, Bolen, and Israel)

Biological and biochemical characterization of lymphotropic papovavirus. The virus-coded proteins of lymphotropic papovavirus (LPV) were determined by *in vitro* translation of LPV mRNA followed by immunoprecipitation with anti-LPV serum. The viral products identified were 81K, 41K, 35K, and 26K proteins. The latter three proteins were also found in purified LPV particles and therefore designated as VP1, VP2, and VP3 capsid proteins, respectively. The 81K protein was an early gene product and, most likely, is the T-antigen analogue of LPV.

Serological surveys have been extended and sera from various types of patients were examined in attempts to associate LPV with specific human disease. A high percentage (26%) of children with acute lymphocytic leukemia were positive for LPV antibody, whereas sera from normal children of comparable age groups were negative. The specificity of LPV antibody was demonstrated by neutralization tests and also by the ability of positive sera to immunoprecipitate the major capsid protein (41K) of LPV. (Takemoto, Segawa, and Kanda)

DNA virus-transformed hamster cell-host effector cell interactions: level of resistance to cytolysis correlated tumorigenicity. Hamster spleen cells and activated hamster macrophages were used to study effector cell susceptibility to lysis among 13 DNA virus-transformed hamster cell lines exhibiting four different tumorigenic phenotypes. Hamster cells transformed by adenovirus 12 (an oncogenic adenovirus serotype) or simian virus 40 (an oncogenic papovavirus) readily induced tumors in immunocompetent syngeneic hamsters and were relatively resistant to lysis by spleen cells while adenovirus type 2 (a nononcogenic adenovirus serotype) transformed cells, which only induced tumors in immunoincompetent hosts, were easily lysed. Simian virus 40 transformed cells, which possess the unusual property of efficient tumor induction in allogeneic hosts, were resistant to lysis by both spleen cells and activated macrophages. Thus, resistance to lysis by activated macrophages discriminates between SV40 transformed hamster cells that express the greatest tumor-inducing capacity for hamsters and adenovirus transformed hamster cells that are less tumorigenic. Resistance to lysis by hamster spleen cells disciminates between adenovirus 12 transformed hamster cells and adenovirus 2 transformed hamster cells. These findings suggest an association between the level of transformed cell resistance to lysis and the oncogenicity of the transforming virus and also suggest that cell transformation and the expression of resistance or susceptibility to immune rejection are independent events. (Cook, Hibbs, Lewis)

Host cell-dependent hybrid DNA arrangement in Ad2^{+}ND viruses. Ad2^{+}ND viruses are characterized by rearrangements in early region 3 (E3). Variable parts of the adenovirus 2 sequences in E3 are deleted, and in their place sequences from the early region of SV40 are inserted. The virus with the largest SV40 insert, Ad2^{+}ND$_4$, may be considered the prototype of the Ad2^{+}ND virus family. Ad2^{+}ND$_4$ adapts to growth in different host cells by deleting sequences at the left Ad2-SV40 junction. Hybrid virions with genomes containing these deletions include the previously described Ad2^{+}ND$_1$, Ad2^{+}ND$_2$, Ad2^{+}ND$_3$, and Ad2^{+}ND$_5$ hybrids.

Two others, $Ad2^{+}ND_{4del}$ and $Ad2^{+}ND_{6}$, have been isolated recently. The genome of $Ad2^{+}ND_{4del}$ is missing that portion of the SV40 DNA segment from map position 0.51 to 0.61 which conveys a selective advantage to $Ad2^{+}ND_{4del}$ virus to grow in human cells, alters its ability to grow in monkey cells, and abolishes its ability to express the serologically detectable SV40 U antigen determinant that is present on SV40 large T protein. A feature common to all deletion variants is the fact that they lack the splice sites for SV40 T antigen mRNA present in $Ad2^{+}ND_{4}$. It appears likely that the elimination of these splice sites is the means by which $Ad2^{+}ND_{4}$ adapts to growth in different host cells. (Lewis, Gerry, and Westphal)

Epidemiology of β-hemolytic Streptococcus faecalis infections. The genetic determinants for plasmid-associated β-hemolysis and bacteriocin production by five clinical isolates of *S. faecalis* were virtually identical by DNA-DNA hybridization and restriction endonuclease analyses. The plasmids were also between 50% and 100% homologous with each other. Preliminary results suggested that three of the isolates may have had a recent common ancestral origin. (LeBlanc and Banai)

Molecular and genetic analysis of plasmid pAMβ1. A detailed restriction endonuclease map (41 sites) of the broad host-range conjugative plasmid, pAMβ1, was constructed. Deletion mapping strategies located the antibiotic resistance, conjugation, and replication functions on the physical map. Appropriate restriction fragments were cloned on *E. coli* and *S. sanguis* vector plasmids for further elucidation of enzymatic activities associated with the conjugative and replicative functions of this plasmid. (LeBlanc and Ranhand)

The role of plasmids in streptococcal carbohydrate metabolism. Transformation of lactose-negative mutants of *S. sanguis* Challis by plasmid DNA from *S. lactis* restored two lactose-specific enzyme activities in the transformants, but no plasmid DNA could be isolated from them. These same functions could also be restored by transformation with plasmid restriction enzyme digests. Cloned fragments from the same preparation of digested plasmid DNA could not restore these functions, suggesting that regulation of the lactose-specific genes required contiguous chromosomal DNA in the transformed Challis isolates. (LeBlanc)

Characterization of a new genital tract mycoplasma. The new mycoplasma (G-37) recovered from two patients with non-gonococcal urethritis has been characterized in a number of morphological, biological, or serological tests. The new name proposed for the organism is *Mycoplasma genitalium*. Experimental challenge studies in chimpanzees show the organism to colonize the urogenital tract for periods of three months or more. (Tully, Taylor-Robinson, and Barile)

Spiroplasmas in central nervous system infections. Further work on the characteristics of the central nervous system disease induced in suckling rats by spiroplasmas (SMCA) has shown vacuolar degenerative changes similar to histological changes observed in some slow degenerative diseases of man. (Tully, Bastian)

Spiroplasma characterization. Serological procedures and some additional techniques have been employed in defining a large number of new spiroplasmas serogroups, representing isolates primarily from a variety of inserts. The identification of these new groups provides representative strains to explore spiroplasma vertabrate interactions and pathogenicity. (Tully, Whitcomb, and Bove)

Honors and Awards

Dr. Malcolm A. Martin continues to serve on the editorial board of the *Journal of Biological Chemistry* and reviewed papers from *Science*, *Nature*, and the *Journal of Virology*. As an Adjunct Associate Professor of Microbiology at the University of North Carolina, Dr. Martin supervised the dissertation research of Ph.D. candidates. Dr. Martin was selected to be a member of a three-person team which comprised the Indo-U.S. Cooperative Exchange Group on Recombinant DNA and gave lectures in Delhi, Bombay, and Bangalore. Dr. Martin continues to serve on the Recombinant DNA Executive Committee. During the year he presented lectures at the University of North Carolina, the Johns Hopkins University, and Howard University.

Dr. Joseph G. Tully received the J. Roger Porter award from the U. S. Federation of Culture Collections, ASM, and the Emory Klieneberger-Nobel Award from the International Organization for Mycoplasmology. He is a member of the Board of Directors, International Organization for Mycoplasmology and serves on the Advisory Committee on Bacterial Collection of the American Type Culture Collection. He was a co-editor, Mycoplasma Symposium, *Reviews of Infectious Diseases*. In May, 1982, Dr. Tully gave invited lectures in Bordeaux, France; Tokyo, Japan; as well as for the American Society for Microbiology annual meeting in Atlanta.

Dr. Kenneth K. Takemoto was an invited lecturer at the 1) First International Symposium on Medical Virology, Anaheim, California, and 2) Symposium on Human Polyoma Viruses and Neurological Diseases sponsored by the Fogarty International Center, Bethesda, Maryland.

Dr. Donald J. LeBlanc was co-chairman of the First International Meeting on Streptococcal Genetics, Sarasota, Florida, and presented an invited talk at the Downstate Medical School, Brooklyn, New York.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00006-11 LMM

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Competence Development and Genetic Exchange Mechanisms Among Streptococci (transferred from LSD)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Jon M. Ranhand	Senior Scientist	LMM	NIAID

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Bacterial Virulence

INSTITUTE AND LOCATION
NIAID, NIH, Ft. Detrick, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Studies designed to understand the mechanism of competence in group H streptococci were continued. Two new relevant observations made were (i) the inhibition of genetic transformation (a measure of competence) by a naturally occurring Gram-positive cell-membrane polymer called lipoteichoic acid (LTA) and (ii) the stimulation of competence factor (CF) activity in cells by a heat (100°C) and proteinase stable component present in a number of normal sera derived from a variety of animals. LTA (1) prevented the development of competence by inactivating CF in an unknown way, and (2) inhibited the development of competence after CF reacted with cells. (2) might reflect the inhibition of a specific competent cell-associated autolysin since it has been shown by others that LTAs and similar related compounds inhibit autolytic activities in a number of bacteria. Cells treated with an elevated serum concentration (12.5% v/v) or exudates from boiled sera during growth were made more sensitive to CF. The nature of the serum factor(s) is unknown. In addition, cells grown in media containing high serum concentrations and then centrifuged reacted better with CF than did controls. It is possible that serum and centrifugation remove naturally occurring LTAs that inhibit CF associated reactions.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00013-19 LMM

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Studies of Viral Antigens in Virus-induced Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Andrew M. Lewis, Jr.	Research Virologist	LMM	NIAID

COOPERATING UNITS (if any)
Arthur Levine and Cephas Patch, NCI, NIH; Heiner Westphal, NICHHD, NIH; James L. Cook, National Jewish Hospital, Denver, Colorado; and John B. Hibbs, University of Utah Medical Center

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Viral Biology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

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3	1	2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The objectives of this project are to use human adenoviruses (Ad) simian virus 40 (SV40) and Ad-SV40 recombinants to define the role of viral genes and viral antigens in tumor induction by DNA viruses and to study the biology of Ad2-SV40 recombinants. SV40 is highly oncogenic for Syrian hamsters; this virus transforms hamster cells in tissue culture and these transformed cells produced tumors when injected into newborn and adult hamsters. Ad2 is nononcogenic for hamsters; this virus also transforms hamster cells in tissue culture but these transformed cells make tumors only in immunologically immature newborn hamsters or athymic nude mice. Studies of the tumor-inducing properties of Ad2- and SV40-transformed cells suggest that SV40 conveys to transformed hamster cells the ability to resist rejection by the hamster's cellular immune system. Ad2 appears to be unable to convey resistance to cellular immune rejection to the hamster cell it transforms. These data imply that, in addition to transformation, the induction of some type of resistance to immune rejection is essential to the process by which SV40 converts normal cells to tumor cells. The incorporation of the segment of the SV40 genome which induces this resistance to immune rejection into Ad chromosomes could explain the mechanism by which SV40 conveys increased oncogenicity to Ad-SV40 recombinants.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00018-16 LMM

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Biological and Biochemical Characterization of Human Papovaviruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Kenneth K. Takemoto	Head, Viral Biology Section	LMM	NIAID
OTHERS:	Tadahito Kanda	Visiting Fellow	LMM	NIAID
	Kaoru Segawa	Visiting Fellow	LMM	NIAID

COOPERATING UNITS (if any)
Kunito Yoshiike, National Institute of Health, Tokyo, Japan

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Laboratory of Molecular Microbiology

SECTION
Viral Biology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2-7/12	1-7/12	1

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies which were initiated last year on the characterization of a new member of the polyomavirus genus, a B-lymphotropic papovavirus (LPV) have been expanded during this past year to include biochemical studies on the viral proteins as well as its DNA. Three viral polypeptides have been identified, both in purified virus preparations as well as in infected cells. These are the major capsid protein, VP-1 (41K), and two internal proteins, VP-2 (35K) and VP-3 (26K). Besides the structural proteins, an early, virus-coded 81K protein was identified in infected cells. The 81K protein may be the large T-antigen of LPV. Investigations on the genome of LPV is proceeding along two different directions. A more detailed physical map is being constructed and, in addition, studies are underway to compare the LPV DNA with the genomes of the other primate polyomaviruses, BK, JC, and SV40.

In biological studies, the prevalence of antibody to LPV in humans has been expanded. We have previously reported that about 25-30% of adults have antibody to LPV. Only 5% of people under 20 yr have been found to be positive for antibody to LPV. Infection therefore appears to occur later in life in humans.

Attempts to transform a variety of human and rodent cells with LPV have been unsuccessful, but these experiments are still continuing.

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PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Basic Studies of Mycoplasmas (transferred from LID)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Joseph G. Tully	Head, Mycoplasma Section	LMM	NIAID

COOPERATING UNITS (if any)
D. Taylor-Robinson, Clinical Research Center, London; M. F. Barile, Bureau of Biologics, FDA; F. O. Bastian, Univ. Maryland; R. F. Whitcomb, Insect Pathology Laboratory, USDA; J. M. Bove, Univ. Bordeaux, France.

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SECTION
Bacterial Virulence

INSTITUTE AND LOCATION
NIAID, NIH, Ft. Detrick, Frederick, Maryland 21701

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3	1	2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) These efforts cover both basic and applied aspects of mycoplasmas and related wall-free prokaryotes, including their neurotoxins, antigens, biologic or genetic factors involved in virulence, immunological interrelationships, and possible role in human disease or diseases of uncertain etiology. Current efforts are directed to the characterization of a newly recovered mycoplasma from the urogenital tract of patients with non-gonococcal urethritis. The unique terminal structure on the organism appears to be involved in attachment of the microbe to erythrocytes, tissue cells, and other surfaces. Pathogenicity studies, utilizing experimental inoculation of organisms into the urogenital tract of a variety of primates, indicates the new mycoplasma can colonize primates for periods of at least 8-9 weeks. Efforts to characterize the pathogenicity and serological properties of an expanding group of helical mycoplasmas (spiroplasmas) have continued. Experimental brain infection of suckling rats (with the GT-48 strain of *Spiroplasma mirum*) yielded correlation between the histopathological changes, numbers of viable organisms in brain tissue, and immunocytochemical localization of spiroplasmas. Infection of the gray matter of brain supports our concept that the spiroplasmas induce responses similar to other slow degenerative brain diseases of man.

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U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00190-04 LMM

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
The Molecular Genetics of Eukaryotic Cells and Their Viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dr. Malcolm A. Martin	Chief	LMM	NIAID
OTHERS:	Arifa S. Khan	Staff Fellow	LMM	NIAID
	Arnold B. Rabson	Medical Staff Fellow	LMM	NIAID
	Paul E. Steele	Medical Staff Fellow	LMM	NIAID

COOPERATING UNITS (if any)
Wallace P. Rowe, Charles E. Buckler, and M. David Hoggan, LVD, NIAID, NIH; George Khoury and Peter Gruss, LMV, NCI, NIH

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Laboratory of Molecular Microbiology

SECTION
Biochemical Virology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7-11/12	3-11/12	4

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The principal aim of these investigations is the study of the molecular structure and genetic regulation of exogenous and endogenous type C mammalian retroviruses. Using techniques of molecular cloning, nucleic acid hybridization and nucleotide sequencing, endogenous MuLV-related proviral DNAs from normal mouse, monkey, and human cells have been extensively characterized. In the mouse system results have been obtained that indicate that 1) the majority of endogenous MuLV envelope (env) sequences are related to neither ecotropic nor xenotropic analogues but appear to be precursors of gene segments encoding MCF MuLV env determinants; and 2) the LTR component of endogenous MuLV proviruses differs from the LTRs of known infectious viruses by containing a unique, highly conserved 105 bp insertion located in the U3 region between the 72 bp direct repeat and the CAAT box. Twenty-nine different MuLV-related segments have been molecularly cloned from human DNA. Nucleotide sequencing studies have shown that the regions coding for pol, and the p30 and p10 gag polypeptides are highly conserved and collinear with those specifying the same gene products in Moloney MuLV.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00217-01 LMM

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Oncogenesis: Molecular Biology of Initiation and Maintenance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Mark A. Israel	Medical Officer	LMM	NIAID
OTHERS:	Kamal A. Chowdhury	Visiting Associate	LMM	NIAID
	Joseph B. Bolen	Staff Fellow	LMM	NIAID

COOPERATING UNITS (if any)
Clyde Dawe, LP, NCI, NIH

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Laboratory of Molecular Microbiology

SECTION
Biochemical Virology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3-11/12	2-11/12	1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

During the past year, our efforts have been directed toward elucidation of the molecular mechanisms underlying the biologic activity of polyoma virus (Py). We are continuing to characterize the viral-host DNA junctions in polyoma virus-transformed cells in order to formulate a role in integration for short areas of homology which we have observed in DNA sequences flanking the site of viral DNA integration into the host cell chromosome. Also, we have identified several species of the Py T-antigens by isoelectric focusing and are currently characterizing these both biochemically and on the basis of their reactivity to monoclonal antibodies. This year we initiated a new line of research to study the molecular basis of Py virus virulence and organ-specific tumorigenesis in newborn mice. Preliminary data indicate that these characteristics are genetically determined by alterations in a region of the genome responsible for the regulation of Py virus early gene expression.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00218-01 LMM

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biochemical and Chemical Studies on Retroviral DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Roy Repaske	Chemist	LMM	NIAID

COOPERATING UNITS (if any)
C. Buckler and M. D. Hoggan, LVD, NIAID, NIH

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Biochemical Virology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2-9/12	1	1-9/12

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Cloned murine leukemia virus (MuLV)-like human genomic DNA segments were sequenced and compared to Moloney (Mo) MuLV proviral DNA. The sequenced human DNA (about 2600 total nucleotides) was analyzed by computer for homology and location within the MoMuLV genome. Regions sequenced corresponded to portions of the gag proteins p12, p30, and p10, and reverse transcriptase (pol). Approximately 400 bases were also sequenced from a cloned fragment of African green monkey DNA which hybridized both to human and Mo-MuLV proviral DNA; sequence homology was also demonstrated.

Another project dealt with two types of mouse MuLVs (ecotropic and xenotropic), which have unique host range specificity. Differences (hybridization) occur in the region encoding for envelope (env) glycoprotein. The sequence of portions of the pol and env regions of xenotropic proviral DNA was determined and compared to the published sequence of analogous segments of the AKR ecotropic provirus. Analyses showed nearly identical homology in the pol region, whereas short stretches of homology and regions of nucleotide deletions and insertions occurred in the env region. No major difference in the physical characteristics of the protein translated from xenotropic env-specific sequences was noted.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00219-01 LMM

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Molecular and Genetic Analysis of Plasmid-Mediated Properties Among Streptococci

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Donald J. LeBlanc	Acting Head, Bact. Virulence Sect.	LMM	NIAID
OTHER:	Menachem Banai	Visiting Fellow	LMM	NIAID

COOPERATING UNITS (if any)
Dr. Don B. Clewell, Dept. of Oral Biology, University of Michigan, Ann Arbor, MI; Dr. Detlev Behnke, Academy of Science, Jena, GDR

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Laboratory of Molecular Microbiology

SECTION
Bacterial Virulence

INSTITUTE AND LOCATION
NIAID, NIH, Ft. Detrick, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2-8/12	1-8/12	1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

During the past year, a retrospective epidemiological study of β-hemolytic, group D, streptococcal infections was initiated. Conjugative hemolysin plasmids were isolated from five clinical Streptococcus faecalis isolates of diverse geographical origins. Cloned DNA, containing the hemolysin gene(s) from one of these plasmids (pAD1), hybridized to the same two Eco RI restriction fragments from each of the five plasmids. Hybridization experiments using total plasmid DNA suggested that the plasmids shared between 40% and 100% homology with each other. Two of the plasmids, pAD1 from strain DS16 and pAMγ1 from strain DS5, were 100% homologous and had identical Eco RI restriction patterns (eight fragments each). Two of the S. faecalis strains, DS16 and JH1, shared a sufficient number of "atypical" properties to suggest a common ancestral origin: 1) their hemolysin plasmids, pAD1 (DS16) and pJH2 (JH1), were at least 90% homologous; 2) each strain contained a chromosome-borne tetracycline resistance transposon with at least partial homology between them; 3) each strain harbored an antibiotic resistance plasmid, pAD2 (DS16) and pJH1 (JH1), mediating resistance to streptomycin, kanamycin, and erythromycin, the latter trait being transposable in each case.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00220-01 LMM

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Structure and Function of the Oncogene Products of Polyoma Virus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Yoshiaki Ito	Visiting Scientist	LMM	NIAID
OTHERS:	Kaoru Segawa	Visiting Fellow	LMM	NIAID
	Mark A. Israel	Medical Officer	LMM	NIAID
	Kamal A. Chowdhury	Visiting Associate	LMM	NIAID

COOPERATING UNITS (if any)
Kei-ichi Tanaka, Kenneth Chang, and Ettore Appella, Laboratory of Cell Biology, National Cancer Institute

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Biochemical Virology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2-10/12	PROFESSIONAL: 1-11/12	OTHER: 11/12

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In our continuing effort to study the structure and function of polyoma virus T antigens, we have found that middle-size T antigen-associated protein kinase can exist as an active or inactive tyrosine-specific protein kinase. The active kinase is present exclusively in the plasma membrane, and appears to be phosphorylated in vivo at serine residues. These results suggest that the activity of "middle T kinase" may be regulated by a cellular plasma membrane enzyme. Antiserum against a synthetic polypeptide representing the region of middle-size T antigen believed to contain the phosphate acceptor site for the in vitro kinase reaction reacts exclusively with middle-size T antigen and blocks its in vitro phosphorylation. Polyoma virus mutants capable of growing in embryonic cells which are resistant to wild-type polyoma virus infection have also been isolated. In each case, a mutation was localized to the portion of the genome which regulates replication and DNA transcription. The ability of these virus mutants to grow in embryonic cells was found to depend on the stage of cellular differentiation.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00222-01 LMM

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Characterization of Endogenous Ecotropic and Xenotropic Murine Leukemia Viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Theodore S. Theodore	Research Microbiologist	LMM	NIAID
OTHER:	Malcolm A. Martin	Chief	LMM	NIAID

COOPERATING UNITS (if any)
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INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1-1/12	1-1/12	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The aim of this project is to study the molecular organization of endogenous retroviruses. Using restriction enzyme mapping techniques, agarose gel electrophoresis, nucleic acid hybridization procedures, and transformation and transfection techniques, we have characterized the organization of endogenous ecotropic and xenotropic murine leukemia viruses in mouse chromosomal DNA. We have isolated, by preparative agar gel electrophoresis, and are cloning, using a cosmid vector system, the endogenous ecotropic proviruses present in AKR/J and BALB/c mouse DNAs. BALB/c mice contain numerous copies of endogenous xenotropic proviral DNAs which are also being molecularly cloned using cosmid vectors.

PHS-6040
(Rev. 2-81)

LABORATORY OF PARASITIC DISEASES

1982 ANNUAL REPORT

Table of Contents

Z01 AI
Project Number

SUMMARY 25-1

00092-16 The Pathogenesis of Schistosome Infections in Mammalian Hosts - Cheever.........................25-14

00094-23 Studies on *Entamoeba histolytica* and other Parasitic Protozoa - Diamond......................25-15

00097-24 Physiological and Cytochemical Pathology of Parasitic Diseases - Mercado......................25-16

00098-26 Biochemical Mechanisms of Energy Metabolism in Mammalian and Parasitic Organisms - Weinbach.......25-17

00099-12 Biophysical Parasitology - Dvorak.................25-18

00102-08 Pathogenesis of Disease Caused by Infection with Intracellular Parasites - Neva.....................25-19

00103-15 Immunological Studies on Toxoplasmosis and Other Parasitic Diseases - Lunde........................25-20

00108-11 Studies on the Biology and Immunogenicity of Malaria Sporozoites - Gwadz........................25-21

00160-06 Cell Biology of Entamoeba, Giardia and Other Parasitic Protozoa - Gillin..................25-22

00161-05 Immunochemistry of Parasitic Diseases - Nash.......25-23

00162-06 Biochemical Cytology of Host-parasite Interactions in Parasitic Protozoa - Dwyer.....................25-24

00185-03 Pathogenic Protozoa: Structure, Virulence Factors and Endogenous Viruses - Mattern..........25-25

00197-03 Immune Recognition in Filariasis and Other Helminth Infections - Hussain.....................25-26

00208-02 DNA of *P. berghei* and *S. mansoni* - McCutchan........25-27

00240-01 Culture Physiology and Antigenic Analysis of Sexual Stages of Malaria Parasites - Carter.........25-28

00241-01 Identification of Receptors of Merozoite Invasion of Erythrocytes - Miller............................25-29

00242-01 Biological and Biochemical Studies of Antigens on Malaria-infected Red Cells - Howard................25-30

00243-01 Characterization of Malarial Proteases: Their Role in Hemoglobin Digestion, Invasion and Rupture - Hadley..................................25-31

00244-01 Developmental Adaptations of *Trypanosoma cruzi* to the Vertebrate Immune System - Sher..............25-32

00245-01 Macrophage Involvement in Immunity to *Schistosoma mansoni* Infection - James...............25-33

00246-01 Molecular Studies of the Genome and Surface of *Schistosoma mansoni* - Simpson.......................25-34

00247-01 Immunochemical Analysis of Cloned *Trypanosoma cruzi* Stocks - Bongertz............... 25-35

00248-01 Physiology and Genetics of Anopheles Mosquitoes - Collins..................................25-36

00251-01 Studies on the Immunologic Basis of Resistance in Mice Vaccinated with Irradiated Cercariae - Sher....25-37

00252-01 Cell Biological Studies of *Trypanosoma Cruzi* Clones Isolated from Chronic Chagasic Patients - Engel.....25-38

00253-01 Studies of the Immunologic Responses to Filarial Infections - Ottesen 25-39

00254-01 Studies of the Host Response in Onchocerciasis - Ottesen...25-40

00255-01 Studies of the Immunologic Responses to Schistosome Infections - Ottesen...............................25-41

00256-01 Regulation of Immunity to Pathogenic Protozoa - Sacks - Scott..25-42

00257-01 Immunology of Strongyloidiasis - Neva..............25-43

00258-01 Development of Stable, Reproducible Models for Chagas' Disease using *Trypanosoma cruzi* Clones and Inbred Mouse Strains - Postan.......................25-44

The following projects have been terminated.

00093-06 Studies of the Immunologic Responses to Helminth Infections - Ottesen.................................25-45

00149-06 Physiology of Anopheles Mosquitoes - Rosenberg......25-46

00174-04 Culture, Physiology and Antigenic Analysis of Sexual and Asexual Erythrocytic - Carter............25-47

00198-02 Host Immunological Response to *Giardia lamblia* Infection - Smith.................................25-48

00207-01 Mechanisms of Immunity and Immune Evasion in Parasitic Infection - Sher.........................25-49

Laboratory of Parasitic Diseases

National Institute of Allergy and Infectious Diseases

SUMMARY - October 1, 1981 - September 30, 1982

INTRODUCTION

Last year the main administrative pertubations involved the move of Dr. Ottesen and his group to the new ACRF Building, as well as the planning to accommodate the Malaria Section in Building 5 as part of the "round robin" of moves required for renovation of NIH buildings. Regretfully, overall the DHHS personnel policies on USPHS officers brought about a sooner than expected retirement of Dr. Carl "Ted" Mattern. Fortunately, he will continue his trichomonas work at Johns Hopkins. Ted Mattern's career at NIH spanned a remarkable array of topics and duties - a USPHS officer "for all seasons". Dr. Frances Gillin, who had become almost a permanent fixture, left for a new job at the University of California in San Diego, where her husband (from NIMH/NIH) also begins a new academic position. The Laboratory of Parasitic Diseases is not only a research laboratory, it also facilitates matrimony, as witnessed by Drs. Deep and Nuzhat (formerly Anwar) Kaushal. The Kaushal's will take a new baby back to India with them as they return to the Drug Research Institute in Lucknow, India. Another man and wife team, Drs. Manoel and Aldina Barral returned to Bahia, Brazil, where they will be working in Professor Zilton Andrade's Department of Pathology. Dr. Mark Hofstetter left for a final year of clinical infectious disease training at the University of Michigan. Dr. Joan Rener has a position with industry in the Washington area.

Two new Guest Workers have joined the Malaria group, Drs. Peter David and Stephen Aley. Dr. James Leech, an Associate from the Laboratory of Clinical Investigations, is also working with the Malaria Section. Two new Associates, or Medical Staff Fellows as they are now called, have joined the LPD; they are Dr. L. Vaughn Kirchhoff, from LCI, and Dr. Thomas Nutman, from a medical residency at N.Y.U. Dr. Haynes "Chip" Sheppard is a Staff Fellow, who came to work with Dr. Dwyer. Several Guest Workers from local institutions are spending time in Dr. Nash's laboratory - Dr. Carmelita Tuazon, who heads the I.D. Division at George Washington University Hospital, and Dr. Correa-Coronas, a Navy Captain from the Naval Medical Center.

As usual, research work continues to be carried out in collaboration with various groups in foreign countries. During Dr. Ottesen's annual period of work in India, he visited the onchocerciasis hospital of Dr. Awadzi in Tamale, Ghana and initiated plans for detailed immunologic studies of the reactions (Mazzotti) that occur after treatment with DEC. Dr. Francis from the LPD then spent about a month in Ghana working with Dr. Awadzi on this project. Dr. Renato Gusmão, as LPD Guest Worker who is a graduate student at Johns Hopkins, spent 6 months in Goiania, Brazil, following up on several aspects of work we had initiated there. Dr. Gusmão will incorporate these studies into his Ph.D. research thesis. Dr. Neva is scheduled to go to Caracas, Venezuela, to work with Dr. Petersen of the University of Arizona and collaborators from Dr. Convit's group at the Institute of Dermatology on cutaneous leishmaniasis.

Laboratory of Parasitic Diseases

National Institute of Allergy and Infectious Diseases

SUMMARY - October 1, 1981 through September 30, 1982

HONORS AND AWARDS

Dr. Louis Diamond was elected President of the Helminthological Society of Washington, served as Coordinator and Conference Leader of the Ninth Seminar on Amebiasis in Mexico City sponsored by several Mexican agencies, and was an invited participant in symposia at national and international meetings.

Dr. Dennis Dwyer was sole recipient of the Alumni Citation Award for 1981-82 given by the Alumni Foundation of Montclair State College of New Jersey. He has appointments as Adjunct Associate Professor at the University of Massachusetts and Rockefeller University, and served as Consultant to the TDR Program of WHO.

Dr. Rabia Hussain was an invited participant by WHO to an international conference in Sri Lanka.

Dr. Stephanie James was an invited participant for the U.S.-Japan Parasitology program held in Japan during the summer of 1982.

Mr. Milford Lunde was elected Vice-president of the Helminthological Society of Washington.

Dr. Teresa Mercado was an invited participant as discussion leader at a session for students sponsored by the Minority Biomedical Support Program of NIAID.

Dr. Louis Miller was invited to give the Lloyd Rozeboom Lecture at Johns Hopkins University and continues as a member of one of the Steering Committees on Malaria of the TDR Program for WHO.

Dr. Theodore Nash received the Commendation Medal of the U.S. Public Health Service in 1982.

Dr. Franklin Neva spent several days as Carl Harford Visiting Professor at Washington University in St. Louis. He continues to hold appointments as Visiting Lecturer at the Harvard School of Public Health and at Johns Hopkins School of Hygiene. He served as Consultant to the TDR Program of the WHO.

Dr. Eric Ottesen was appointed Chairman of the Scientific Working Group on Filariasis for the TDR Program of the WHO.

Dr. Alan Sher was asked to help organize and participate in one portion of the program for the American Association of Immunologists annual meeting, and gave a number of invited lectures at different institutions.

Dr. Eugene Weinbach organized and participated in a special Symposium at the International Congress of Parasitology honoring the late Dr. Theodore von Brand.

Laboratory of Parasitic Diseases

National Institute of Allergy and Infectious Diseases

SUMMARY - October 1, 1981 through September 30, 1982

RESEARCH ACCOMPLISHMENTS

MOLECULAR BIOLOGY

PARASITES

Characterization of schistosomal genome: The genome size (2.7 x 10^8 base pairs) and repetition pattern OF of S. mansoni DNA has been determined, and cloning and mapping of the parasite's ribosomal RNA (rRNA) has been carried out. When fragments of rRNA are hybridized to Southern blots of restricted genomic DNA, a single major band results, but the pattern of minor bands of hybridization permit not only species but even strains within the species to be distinguished. Thus, these techniques may be useful in studies of schistosome genetics and taxonomy (Simpson and McCutchan). By genomic hybridization studies no support was found for the interesting hypothesis that the schistosome genome might code for snythesis of mouse or human histocompatibility antigens to explain their presence on the worm surface (Simpson, Sher, McCutchan).

Surface polypeptides of schistosomes as immune targets: Since the schistosome becomes totally refractory to immune effector mechanisms within 5 days after entry into the vertebrate host, changes in the parasite surface at the molecular level may illuminate the mechanisms of immunity to the parasite. Ten major polypeptide components were identified by external labelling with ^{125}I. Comparison of the immunoprecipitation patterns in mice that develop good immunity versus those that develop poor immunity after infection, as well as in mice immunized with irradiated cercariae, suggest that small molecular weight components of the parasite are the main protective antigens. Another approach to identification of surface antigens on the parasite as it develops is by lectin binding, and with monoclonal antibodies against mouse major histocompatibility antigens. These studies have shown that mannose and/or glucose, galactose and N-acetyl-glucosamine are the exposed saccharides on the parasite surface membrane, and that by the time the parasite reaches the lung, the lectin binding pattern is quantitatively greatly reduced and sialic acid begins to increase (Simpson and Sher).

Nucleic acids and genes of malarial parasites: Malarial parasites are particularly interesting organisms for DNA research because they have a well-known but variable biology, and because they alternate between a haploid and diploid genome in their asexual and sexual cycles, respectively. In addition, possible changes in gene expression can be sought in the different life cycle stages. In contrast to other eukaryotic organisms, Plasmodia were found to have only 4 ribosomal genes, not in any recognizable tandem array, and not amplified during their life cycle (McCutchan). Fragments of genomic

DNA from *P. berghei* have been cloned in a phage and plasmid for use as hybridization probes to map gene arrangement. This mapping of rRNA genes of the parasite will be continued and compared to *P. gallinaceum* (Dame and McCutchan).

Receptors and antigens on malaria-infected red cells: Since his demonstration 7 years ago that a receptor on the red cell controls invasion by the parasite, Dr. Miller has continued to make slow but steady progress in defining the molecular nature of the receptor. By use of a monoclonal antibody, band 3, a major transmembrane protein of red cells, has been identified as a receptor for *P. knowlesi* in one phase of the invasion process (Miller). An apparent paradox that has stymied attempts to characterize surface components of the merozoite involved in receptor recognition may finally be resolved. All monoclonal antibodies to the merozoite surface immunoprecipitated a large 230,000 MW glycoprotein of the parasite that had been biosynthetically labelled. But no such molecule existed on surface-labelled merozoites. Instead the monoclonal antibodies precipitated smaller MW proteins. This, plus other evidence suggests that the 230,000 MW protein is present in the schizont or on the red cell membrane, but is enzymatically cleaved just before the merozoites are released (Miller and Howard). The variant antigen on the surface of *P. knowlesi* infected schizonts has finally been isolataed. This, too, required some technical innovations before it could be done - (1) the laborious preparation of cloned parasites with defined and stable variant phenotype and (2) the realization that cell surface radiolabelling methods which were surface specific with uninfected red cells would penetrate the membrane of schizont-infected red cells. The experience with this system should be very helpful in work on antigens and surface components of *P. falciparum* (Howard and Barnwell).

LUMINAL PROTOZOA

Biology and immunology of Giardia The variable outcome of infection with giardia, and the fact that presence of serum antibody alone is not protective indicate that host factors or pathogenic mechnisms as yet unknown are important determinants of disease caused by this parasite. Since human milk is rich in secretory IgA and contains non-specific anti-bacterial factors, milk was examined for its effect on *Giardia in vitro*. Normal human milk (NHM) in a concentrataion as low as 0.3%, but not cow's or goat's milk was found to kill trophozoites in 3-4 hours. The effect was not due to IgA, and was reversed by serum or serum albumin. The giardia-cidal activity of NHM is due primarily to a thermolabile factor, but also contains a less active heat-stabile factor. Several types of evidence suggest that the thermolabile factor is an unusual lipase that resists gastric acid. While the resistant cysts which initiate giardia infection would not be affected by NHM, it could certainly affect trophozoites in the upper small bowel (Gillin).

Surface proteins and antigens of Giardia: The recent ability to grow *Giardia* in culture has now made it possible to label and study antigenic products of the organism. By careful labelling with lactoperoxidase or IODOGEN, the organisms remain viable, multiply and release aboaut 70% of the labelled material which can be immunoprecipitated with anti-giardial antibody. Most of the label seems to be associated with membrane material (Nash). When human strains of *Giardia* from different geographic areas were compared by crossed immunoelectrophoresis clear evidence of antigenic differences was found (Nash, Gillin and Kaushal).

Growth and energy metabolism of Giardia and amebae: The same medium that has been developed for axenic cultivation of *E. histolytica* is basically the same as one which gives optimal growth of *Giardia*. Since LPD has a large background of information concerning nutrition and metabolism of amebae, comparative studies with *Giardia* are particularly fruitful. For example, both organisms contain non-heme iron sulfur (Fe.S) proteins that function in electron transfer. In amebae the ferredoxin type protein has a low redox potential, but in *Giardia* the FeS center has a high redox potential. This finding, plus other evidence, suggests that the electron carrier in *Giardia* is analagous to cytochrome. The plasma membrane of *Giardia* shows evidence of an active proton pump. The organism also contains calmodulin and a CA^{++} activated ATPase, the latter enzyme probably regulated by the calmodulin (Weinbach). A mass cultivation apparatus has been devised for *Giardia*, using a Millipore Pellicon system, which yields up to 9 ml of packed cells in a single harvest (Wieder and Keister). Technical innovations continue to be made in using completely defined media for axenic cultivation of *E. histolytica*. A mixture of nucleotides, vitamins and bases was able to substitute for yeast extract, and purified crystalline bovine albumin could replace the bovine serum component. However, some essential component of either yeast extract or serum, or both, is still needed because the defined replacements cited above will not support continued growth when used together (Diamond). The newborn mouse, as reported by others, was found to be susceptible and to undergo a self-limited infection with human *Giardia*. This may be a useful host to initiate growth of new isolates (Keister).

Metronidazole resistance of human trichomonas: The issue of metronidazole resistance by *T. vaginalis* is still controversial, partly because of rather complicated *in vitro* techniques for testing. Organisms considered to be resistant must be tested under only partially anerobic conditions; under totally anerobic conditions they are not resistant to metronidazole. Five isolates of *T. vaginalis* considered to be drug-resistant were found to show progressive decreases in MLC with 4 additional days of exposure to the drug. Growth of the organisms in ascorbic acid reduced the MLC of highly resistant strains, presumably because of increased anerobiasis. Even though metronidazole resistance is difficult to define and to study, it clearly is an existing phenomenon and promises to become an increasing problem (Mattern).

MALARIA RESEARCH

Sexual stage in mosquitoes: A closer focus upon events in the sexual stages of malaria parasites in the mosquito is useful when considering immunity that could operate at this point in the cycle, as well as when considering genetically resistant vectors. Since LPD workers have been exploring immunity to transmission, an analysis has been made of the antigens found on the stages present in the mosquito gut, and which antigens are the best targets of transmission blocking immunity. With the *P. gallinaceum* -- *A. aegypti* system, both male and female gametes, as well as early fertilized zygotes share a large MW antigen (230 KD) that reacts with monoclonal blocking antibodies. These forms can be lysed in the presence of specific antibody and complement. During development from zygote to ookinete, virtually all surface antigens disappear and a new major antigen of about 30 KD appears on the ookinete. Immune serum raised against ookinetes precipitate the 30 KD antigen and block transformation of zygotes to infective ookinetes, but complement is no longer active against this stage (Carter and Kaushal). A genetic block to parasite development, which occurs at the oocyst stage is being studied using an African vector, *A. gambiae*, and the simian parasite, *P. cynomolgi*. Lines of *A. gambiae* has been selected which are totally refractory or totally susceptible to the parasite. Preliminary evidence indicates that the resistance extends to other species of *Plasmodia*. Therefore, this system appears to be very worthwhile with which the basis for resistance, presumably genetic, can be worked out (Collins and Gwadz). The entomology group are engaged in various collaborative efforts with other investigators by virtue of being able to produce large numbers of malaria-infected mosquitoes (i.e., sporozoites). For example, the N.Y.U. group are provided material for their sporozoite vaccine work and others are studying the latent relapsing EE stages in primates which they have termed hypnozoites. But, the most recent exciting development is the prospect of being able to identify malaria-infected mosquitoes with monoclonal antibody to sporozoites in a radioimmunoassay. If early results on laboratory experimental material can be verified in the field, it would have great practical utility for epidemiologic and malaria control work (Gwadz and collaborators). Although the project does not involve the mosquito vector, the property of gametocyte production in culture by cloned lines of *P. falciparum* is under study. Several other markers that can be utilized in genetic studies, such as isoenzyme type, sensitivity to several different anti-malarial drugs, and endothelial binding are also being followed. It has already become clear that isolates of malarial parasites may consist of heterogeneous mixtures and that the clones appear to be stable for their markers (Graves and Carter).

Binding and "knobs" of P. falciparum: Sticking of *P. falciparum* schizonts to walls of small blood vessels, by knob-like protusions of the red cell surface, is believed to be one of the important properties that accounts for lethal outcome of infection with this species of parasite. What appears to be a convenient *in vitro* model of this phenomenon - namely, binding of cultured red cells infected with mature *P. falciparum* to human endothelial cells *in vitro* has been studied further. An improved binding assay was found using a melanoma cell line instead of umbilical vein endothelial cells, and chronic immune sera reversed the binding of infected cells. Different isolates of *P. falciparum* from various areas of the world, even if they have knobs by EM, show a variable spectrum of binding ability. (Udeinya and Miller) Biochemical and physiological conditions of binding such as Ca^{++} and Mg^{++} requirements, influence of chelators and enzymes, etc., for clues to the nature of surface determinants responsible for binding melanoma cell lines, made available by Sloan-Kettering workers, showed a spectrum of 0 to 100% binding. A series of monoclonal antibodies to different melanoma surface antigens had no influence on binding. Thus, the receptor on melanoma cells to which *P. falciparum* infected red cells attach, remains an elusive but worthy objective of continued search (Leech, Miller and collaborators). There appear to be similarities between the phenomenon of *P. falciparum* binding and the schizont agglutination (SICA) story that occurs with *P. knowlesi*. The latter, SICA in the presence of immune serum, is an expression of antigenic variation that requires presence of the spleen in the infected rhesus monkey. *P. knowlesi* loses its ability to undergo antigenic variation and fails to agglutinate in immune serum - i.e., become SICA negative when passaged in splenectomized monkeys. It has now been found that *P. falciparum* parasitized red cells from splenectomized monkeys fail to bind to melanoma cells and do not agglutinate with immune serum. Incidentally, the switch from SICA+ to SICA- in splenectomized monkeys was accomplished by cloned parasites, to rule out selection of subpopulations (Barnwell).

Biochemistry and cell biology of malaria parasites: Differences have been found in the proteolytic enzymes of the chloroquine-resistant *P. berghei*, as compared to a chloroquine-sensitive (CS) strain. If similar differences are found in CR and CS strains of *P. falciparum*, a very promising avenue of investigation into chloroquine resistance of malaria will have been opened. Encouraging evidence has been found for a protease of merozoites of *P. knowlesi*, which may play an important role in invasion of the red cell (Hadley and Friedman of NIDK). Procedures for isolation and separation of parasite membranes from asexual stages are being developed (Aley).

OTHER INTRACELLULAR PROTOZOA

Studies on leishmania and leishmaniasis: Many aspects of leishmanial infections, including molecular and cell biology of the organism, immunology and clinical features are under investigation in LPD. Major emphasis of the biochemical work continues to be on parasite surface membranes. Since the 3'-nucleotidase is unique to the parasite and not present in vertebrate cells, quantitative activity of the enzyme might reflect numbers of parasites in the tissues. The soluble, extracellular acid phosphotase seems restricted to visceral strains of parasite, so it may be a useful taxonomic feature (Gottlieb and Dwyer). Antibodies to about 10 glycoprotein antigens of the parasite surface membrane can be detected in patients and in experimental animals infected with *L. donovani* (Dwyer). The pattern of cross-reacting proteins and glycoproteins on externally labelled surface membranes of different isolates of *L. tropica* show a number of common antigenic determinants. The crossreactivity extends to the African trypanosome, *T. rhodesiense*, and this latter organism also has a 3'-nucleotidase, similar to the enzyme found in *L. donovani* (Gardiner and Dwyer). A beginning has been made to identify genes from mouse T-cells that code for selected leishmanial antigens (Sheppard and Dwyer). Genetic in-bred strains of mice have become widely used as experimental models for leishmaniasis in recent years because their immunologic responses seem to reflect those of the human host. A dozen or more isolates from well-characterized patients produce a spectrum of disease responses in BALB/c mice inoculated into the foot-pad (Neva). One isolate from Brazil produces a late ulcerative lesion in C57Bl/6 mice, in spite of the development of specific delayed hypersensitivity, in a manner that resembles mucocutaneous leishmaniasis in man (Barral, Sacks, Petersen and Neva). Although most investigators have focused almost exclusively upon cell-mediated immunity as the critical host response in leishmaniasis,--suppressed BALB/c mice exhibited dramatically enhanced resistance to infection with L. tropica and L. mexicana (Sacks and Asofsky). The finding of others was confirmed that non-specifically stimulated lymphokines (LK) can kill or inhibit leishmania in mouse macrophages, and the assay system was refined. However, different strains of parasites react differently - some being quite resistant to LK, and test conditions for LK activity can modify the results in a fashion that probably has clinical relevance (Scott).

Studies on T. cruzi and Chagas' disease: The biologic implications of the population diversity that can exist among a "strain" of T. cruzi parasites has expanded with furher work on clones derived from a single organism. The flow cytometric method of analysis for total DNA per organism has been well standardized. The interclonal differences in total DNA were confirmed by quantitative fluorescence microscopy, and found to involve both nuclear and kinetoplast DNA, with the major contribution to differences due to the nucleus (Dvorak). The growth kinetics of different clones remained stable on passage, and no correlation was found between kinetics of growth and peak modal volume of the clones as determined by Coulter counter (Engel). While an array of cross-reactive antigens was found among a group of parsite clones, some clones may have distinct antigens when tested by crossed line immunoelectrophoresis (Bongertz). But, perhaps the most striking evidence for biologic differences in clones are the pathologic findings in mice that occur after infection with two clones derived from the same "strain" of T. cruzi. One clone (X10/7) produces a rapidly fatal outcome in mice with high parasitemia, whereas the other clone (X10/4) produces in the same strain of mice an infection with very low peripheral parasitemia, and late deaths that feature impressive cardiac lesions (Postan). Transformation of epimastigotes to complement-resistant vertebrate forms of T. cruzi by chamber implantation into mice has been studied further. By parasite irradiation experiments it was shown that the parasites must begin to multiply in order to transform, and that the signal for transformation is acquired after 6-7 hours of implantation. The loss of ability to activate the alternate complement pathway occurs, and the morphologic changes of transformation to trypomastigotes will take place even if the organisms are removed from the Millipore chambers. Surface membrane changes which accompany the transformation process are being studied by lectin binding and with monoclonal antibodies (Sher). The anti-trypanosomal factor (ATF) produced by Pseudomonas bacteria does not seem to be dependent upon endotoxin as judged by the Limulus test, loses its activity after pronase treatment, and is active at a concentration of about 50 μg of protein per ml (Mercado).

Anti-idiotype immunity to African trypanosomes: Further information is now available on the immunity provided to mice against *T. rhodesiense* by administration of anti-idiotype antibodies. Antiidiotypic antibodies were produced against 3 monoclonal antibodies, each with specificity versus a variable antigenic type of the parasite. When pooled IgG, fractions of the anti-idiotypes were given to mice before challenge by the parasite, 19 of 30 animals showed alteration of the course of parasitemia by complete protection, reduced parasitemia, or change in the antigenic type of parasite that appeared (Sacks and Esser of Walter Reed).

HELMINTHIC INFECTIONS

Experimental immunology of schistosome infections: The ability of activated macrophages to kill schistosomula of *S. mansoni* can be non-specifically induced by immunization with BCG and *C. parvum*, and is non-specifically active against murine tumor cells as well. In both instances the activation is dependent upon T-cells, presumably due to lymphokine production by sensitized T-cells. Specifically activated macrophages can also be elicited in *S. mansoni* infected mice and mice infected with irradiated cercariae (James). Various strains of inbred mice have been screened for resistance to challenge after immunization with irradiated cercariae. Reductions in worm recovery varied from <25% to 60-80% among mouse strains, with evidence that high resistance strains are influenced by MHC status (Sher and James). Different strains of infecting parasites, as well as strains of host mice can be examined for ability to induce resistance to reinfection. Such genetic stocks of parasites are available from previous work of Dr. Richards on snail and parasite genetics. These attempts have been plagued by inconsistency in results on repeated experiments, but the evidence appears to show significant differences in ability of some parasite strains to induce resistance to infection (Cheever).

Pathogenesis of disease due to helminths: The mechanism(s) ultimately responsible for liver fibrosis, the main lesion in hepatosplenic schistosomiasis, remains elusive. Tissue collagen is measured by determination of hydroxyproline content, a reasonable indicator of fibrosis. Even though the liver lesions of schistosome infected mice show important differences from human hepatosplenic disease, the mouse may provide useful clues as to how fibrosis is initiated. Different strains of mice were found to up to 4-fold differences in hepatic hydroxyproline per unit number of eggs. Yet, these differences did not correlate with size of granulomas around eggs. The hepatic fibrosis associated with *S. japonicum* infection in rabbits comes close to the periportal fibrosis seen in human infections, but has an unusual degree of reversibility that may be due to the high levels of collagenase in the liver (Cheever). Detection of schistosome antigens in the circulation, tissues or urine has been an important but controversial issue in relating immunopathology and intensity of infection to pathogenesis of disease. Studies with two polysaccharide antigens from adult shcistosome worms illustrate some of the problems involved in interpreting results on fate and clearance of these antigens. One radiolabeled antigen was cleared more quickly from the circulation, with antibody level being the determining factor in speed of clearance. A similar but different antigen, with a liver cell mediated (galactose) clearance mechanism, was actually cleared more slowly from the circulation in the presence of antibody (Nash). An ELISA assay to measure immune complexes in the serum that bind to Clq has been developed and was found to compare very well with a radioimmunoassay (Lunde).

Immune response and regulation: Many different aspects of immediate hypersensitivity (allergy) associated with filariasis and schistosomiasis are under investigation. The filarial system (B. malayi) has made it possible to demonstrate antibodies to several different types of parasite antigens, such as somatic or excretory-secretory (ES), as well as to antigens from different stages of the parasite, such as microfilariae (mf) or adult worms. For example, of some 35 antigens demonstrable by crossed immunoelectrophoresis in adult filarial worms, only 9 of these are parasite-derived ES antigens by autoradiography. Cross-reactivity of IgG antibodies in patients with other infections to various filarial antigens was studied by use of a Staph-A radioimmunoassay (N. Kaushal, Hussain, Ottesen). Parasite-specific IgE can now be quantified in various patient groups by solid-phase radioimmunoassay. The concentration of serum protein that is IgE in usual allergic disorders was about 0.12 to 6 µg per ml, in parasitic diseases it ranged from 1 to 100 µg per ml. In filarial infected populations the mean IgE levels are lowest in those with microfilaremia and highest in the group with tropical pulmonary eosinophilia--i.e.--without circulating microfilariae (Hussain & Ottesen). The opposite relationship applies, as reported last year, to levels of "blocking antibody"--i.e.--IgG which blocks parasite antigen induced histamine release. Since parasite antigens can now be better characterized, and the role of IgE in immediate hypersensitivity (IH) is known, it seems worthwhile to re-explore IH skin tests for diagnostic purposes. This is being done with the Swada skin test antigen (Francis & Ottesen). Incidentally, blocking antibody does not appear to be the cause of falsely negative skin tests in schistosomiasis; they seem due to low or no specific IgE. The possibility that IH mediators may influence delayed hypersensitivity reactions was shown by the fact that histamine released in lymphocyte cultures was capable of suppressing lymphocyte proliferation (Hoffstetter & Ottesen).

Studies on strongyloidiasis: Experience with the ELISA test for serodiagnosis of human strongyloidiasis has been expanded and continues to show antibodies in 85-90 per cent of parasitologically positive cases. Reactivity of periperal blood lymphocytes to S. stercoralis larval antigen in infected individuals is highly variable and not useful in assessment of immune status. However, all infected cases consistently exhibit IgE sensitized basophils by the histamine release test when exposed to strongyloides antigens. Experimental infection of Patas monkeys with human S. stercoralis often leads to fatal outcome and appears to be the only useful model for hyperinfection syndrome with the parasite (Neva, Gam, Genta and Harper).

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00092-16 LPD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Pathogenesis of Schistosome Infections in Mammalian Hosts

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. W. Cheever	Assistant Chief	LPD, NIAID
Others:	T. E. Nash	Senior Investigator	LPD, NIAID
	E. A. Ottesen	Senior Investigator	LPD, NIAID
	F. A. Sher	Senior Investigator	LPD, NIAID
	M. N. Lunde	Research Zoologist	LPD, NIAID
	M. Hofstetter	Research Associate	LPD, NIAID
	R. H. Duvall	Bio. Lab. Tech. (Micro.)	LPD, NIAID
	T. A. Hallack, Jr.	Bio. Lab. Tech. (Gen.)	LPD, NIAID

COOPERATING UNITS (if any) Laboratory Statistics & Mathematic Methodology, Division Computer Research & Technology, NIH (J. Malley); Department of Gastroenterology, Walter Reed Army Institute of Research (M. A. Dunn); Biomedical Research Institute, Rockville, MD (C. S. Richards).

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Host-Parasite Relations Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3.0	PROFESSIONAL: 1.0	OTHER: 2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Schistosome infections are studied quantitatively in man and in experimental animals. Studies in progress are in the following areas: fibrosis: Mouse strains develop markedly different degrees of hepatic fibrosis following infection with S. mansoni. Neither hepatic fibrosis nor other histologic parameters correlated with H-2 haplotype of the strains tested, including a limited number of congenic strains. In a single cross between strains showing high and low fibrosis, fibrosis, was low. Backcross experiments are in progress. Hepatic collagen content correlated directly with collagen synthesis in vitro.

Extracts of hepatic granulomas from schistosome-infected mice stimulate fibroblast proliferation in vitro, but extracts from animals enroute to high or low fibrosis stimulate the fibroblasts equally. Resistance to infection: Using substrains of S. mansoni developed by Dr. Richards, we are investigating the resistance of mice to second infections. Two strains derived from the same patient, PRT-3 and PRC-3, induced markedly different degrees of resistance to reinfection after a bisexual first infection. PRT-3 induced excellent resistance to all challenge strains and PRC-3 induced poor resistance to all strains tested.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00094-23 LPD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies on Entamoeba histolytica and other Parasitic Protozoa

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. S. Diamond	Chief, Parasite Growth and Differentiation Section	LPD, NIAID
Others:	F. D. Gillin	Senior Staff Fellow	LPD, NIAID
	L. Jacobs	Scientist Emeritus	NIAID
	D. B. Keister	Biologist	LPD, NIAID
	C. F. T. Mattern	Medical Officer	LPD, NIAID

COOPERATING UNITS (if any)

American Type Culture Collection, Rockville, MD (Daggett, P.-M.)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Parasite Growth and Differentiation Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 4.0	PROFESSIONAL: 1.3	OTHER: 2.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Two of three undefined components of TYI-S-33, a standard medium used for axenic cultivation of Entamoeba histolytica, have been replaced with defined nutrients. Minimal requirements of the parasite for nucleic acid precursors and B vitamins have been determined using the semi-defined medium. Strains of T-cell deficient and macrophage deficient mice have been screened as potential models for the study of parasite clearance in Giardia lamblia infections. No correlation has been found between ability to clear the parasite and specific immune deficiencies. A new hamster model using animals less than a week old has been developed to study the influence of iron on the production of hepatic lesions in amebiasis. The young animals are more sensitive to infection than the formeraly employed adult animals and can be inoculated percutaneously, obviating the need for a laparotomy. Freeze-preservation of a variety of lumen dwelling protozoa in liquid nitrogen has been facilitated through refinement of controlled-rate and non-controlled-rate freezing techniques.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00097-24 LPD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Physiological and Cytochemical Pathology of Parasitic Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. I. Mercado	Research Physiologist	LPD, NIAID
Others:	A. Colón-Whitt	Microbiologist	LPD, NIAID
	J. K. Inman	Research Chemist	LI, NIAID
	V. Ferrans	Chief, Ultrastructure Section	PB, NHLBI
	J. Butany	Guest Worker	PB, NHLBI
	H. D. Hochstein	Chief, Biological Testing and Reference Standards Branch	DCA, BOB
	K. G. Rice	Research Chemist	LC, NIADDK
	M. Rafferty	Guest Worker	LC, NIADDK

COOPERATING UNITS (if any)
Laboratory of Streptococcal Diseases, NIAID
Laboratory of Immunology, NIAID
Pathology Branch, NHLBI
Biological Testing and Reference Standards Branch, BOB

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Physiology and Biochemistry

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies on the characterization of the anti-trypanosomal factor (ATF) produced by Pseudomonas fluorescens against the Tulahuen strain of Trypanosoma cruzi disclosed further the occurrence of two active small components, ATF II and ATF-III, which exhibited approximate molecular weights of 1,355 and 1,060 when chromatographed with Sephadex G-25. Gel filtration with Sephadex G-200 disclosed that the large active component previously described (ATF-I) exhibited a molecular weight higher than 400,000. The most active fraction was ATF-II, the least, ATF-I. In vitro studies with cultured fibroblasts revealed that the ATF-III produced significant alteration of flagellate populations and amastigote multiplication. Although ATF-II-induced lysis was produced in vivo, these observations indicated that the reduced parasitemia occurring in the ATF-II-treated mice and the control of the acute infection was associated also with an interruption of the parasitic cycle at the tissue level.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00098-26 LPD

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biochemical Mechanisms of Energy Metabolism in Mammalian and Parasitic Organisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. C. Weinbach	Head, Physiology and Biochemistry Section	LPD, NIAID
Others:	D. Dwyer	Research Microbiologist	LPD, NIAID
	C. E. Claggett	Biomedical Laboratory Technician (Biochem.)	LPD, NIAID
	S. Wieder	Medical Technologist	LPD, NIAID
	D. B. Keister	Research Microbiologist	LPD, NIAID

COOPERATING UNITS (if any) Laboratory of Chemical Physics, NIADDK (H. Kon); Clinical Neuropharmacology Branch, NIMH (J.L. Costa); Metabolism Branch, NCI (D. Tschudy and P. Ebert); Dept. of Biochem., Univ. of Stockholm (L. Ernster and Staff)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.10	1.02

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Studies of aerobic energy metabolism have identified the following electron carriers in the respiratory chain of Giardia lamblia and Entamoeba histolytica: pyridine nucleotides NAD(P)H, flavins, and non-heme Fe.S centers. Striking differences in the respiratory metabolism of the two organisms are shown by subcellular distribution and redox properties of the Fe.S centers. These carriers are localized in the particulate fraction of G. lamblia, and organism most intensely studied at present. Calmodulin, and a proton pump related to energy conservation has been found in the plasma membrane of this parasite. Mass cultivation of G. lamblia developed in our laboratory, facilitates studies of the novel findings. Collaborative studies show that kinetoplasts of Leishmania donovani promote the hydrolysis of ATP, and exhibit active respiration which responds differently to inhibitors of energy metabolism than their mammalian counterparts. Studies of mammalian mitochondria have shown that psychoactive drugs (reserpine) have unusual uncoupling properties of oxidative phosphorylation. Other studies of the effect of heme depletion in cultured murine erythroleukemic cells and rat hepatocytes disclose marked differences in their respiratory metabolism.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00099-12 LPD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biophysical Parasitology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. A. Dvorak	Research Microbiologist	LPD, NIAID
Others:	V. Bongertz	Visiting Fellow	LPD, NIAID
	M. Postan	WHO Fellow	LPD, NIAID
	J. C. Engel	WHO Fellow	LPD, NIAID
	P. Engel	Guest Worker	LPD, NIAID

COOPERATING UNITS (if any)

Applied Clinical Engineering Section, BEIB, DRS (T. E. Hall)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Physiology and Biochemistry

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.6	1.6	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project involves basic studies of Trypanosoma cruzi, the obligate intracellular protozoan parasite that causes Chagas' disease. A major goal is an elucidation of the genetic diversity of the species and the resultant biological expression and implications of this diversity. Consequently, major emphasis has been placed on studies of single-cell-isolate clones of the parasite. It has been established that single "strains" of T. cruzi isolated directly from patients are composed of a diverse population of organisms with significant differences in their extra- and intracellular growth rates, transformation potential, total DNA/organism, G1, S and G2-M phases during growth, antigenic composition, course of infection in inbred mice and ability to infect cells in vitro. This is the first multiparametric demonstration of the innate pluripotentiality of natural T. cruzi populations.
N-acetyl-glucosamine has been shown to be the major cell surface monosaccharide in the vertebrate cell receptor complex which influences infection by T. cruzi. None of the monosaccharides which are common constituents of vertebrate cells glycoproteins influence infection by Toxoplasma gondii.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00102-08 LPD

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Pathogenesis of Disease Caused by Infection with Intracellular Parasites

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. A. Neva	Chief	LPD, NIAID
Others:	D. Sacks	Staff Fellow	LPD, NIAID
	A. Gam	Bio. Lab. Tech.	LPD, NIAID
	P. Scott	Staff Fellow	LPD, NIAID
	R. Gusmão	Guest Worker	LPD, NIAID

COOPERATING UNITS (if any) Univ. of Arizona, Tucson, AZ (E. Petersen); Johns Hopkins, Baltimore, MD (R. Gusmão); Inst. of Dermatology, Santa Domingo, D.R.(H. Bogaert); Dept. of Microbiol. & Immunol., Duke Univ. NC (F.E. Ward); Faculty of Med., Fed. Univ. of Goias, Goiania, Goias State, Brazil (J. Rezende & A. Rassi)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Cell Biology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	.7	.9

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A total of 15 isolates of leishmania, most from patients seen or studied at NIH with cutaneous disease have been characterized as to biologic behavior in BALB/c mice. Many of these isolates have also been characterized by recent biochemical taxonomic methods. In the BALB/c mouse these isolates demonstrate a spectrum of biologic response, from failure to persist at the site of inoculation, to metastasis, visceralization and death. These well-characterized strains provide a useful resource for collaborative studies with others on temperature sensitivity, attack by lymphokines, models of muco-cutaneous disease, etc. Additonal experience was gained with several patients using local heat for treatment of cutaneous leishmaniasis. The project on Chagas' disease progressed only with respect to (1) HLA tissue typing that failed to indicate an association with disease and (2) a further inquiry into the significance of humoral suppression of lymphocyte proliferation to T. cruzi antigen. The plaque characterization of T. cruzi strains was temporarily halted.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00103-15 LPD

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immunological Studies on Toxoplasmosis and Other Parasitic Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. N. Lunde	Research Zoologist	LPD, NIAID
Others:	E. A. Ottesen	Senior Investigator	LPD, NIAID
	T. E. Nash	Senior Investigator	LPD, NIAID
	Leon Jacobs	Scientist Emiritus	NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.4	PROFESSIONAL: 1.0	OTHER: 0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Antigenic differences between the cystozoite and endozoite of Toxoplasma gondii have been found. Using fluorescent antibody antisera against the cystozoite reacted positive against only the cystozoite. Antisera against the endozoite reacted positive against both the endozoite and cystozoite. Absorption of sera with endozoites removed only positive reactions with endozoites. These findings are the first to suggest antigenic differences between the zoites within the cyst and zoites outside the cyst of toxoplasma.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00108-11 LPD

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies on the Biology and Immunogenicity of Malaria Sporozoites

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. W. Gwadz	Research Entomologist	LPD, NIAID
Others:	F. H. Collins	Staff Fellow	LPD, NIAID

COOPERATING UNITS (if any)

NYU School of Medicine, New York, NY; (R. Nussenzweig, V. Nussenzweig and A. Cochrane)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.6	PROFESSIONAL: 1.0	OTHER: 1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER
X

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A radio-immunoassay (RIA) has been developed which can detect and identify malaria sporozoites in wild-caught mosquitoes. The presence, quantity and species of malaria parasite can be determined in dead mosquitoes stored for months at room temperature. This assay should become an important tool in investigations of malaria epidemiology in support of vector control programs or vaccine trials.

Monkeys can be immunized with radiation-attenuated sporozoites of Plasmodium knowlesi. Hybridoma produced monoclonal antibodies as well as serum from immunized animals have been used to identify a single protein (MW 42000) on the sporozoite surface which appears to act as the protective antigen.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00160-06 LPD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Cell Biology of Entamoeba, Giardia and Other Parasitic Protozoa

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. D. Gillin	Senior Staff Fellow	LPD, NIAID
Others:	L. S. Diamond	Head, Parasite Growth & Differentiation Section	LPD, NIAID
	T. E. Nash	Medical Officer	LPD, NIAID
	P. D. Smith	I.P.A. Researcher	LPD, NIAID
	F. A. Sher	Head, Cell Biology & Immunology Section	LPD, NIAID
	M. Suffness	Head, Plant and Animal Products Section, Natural Products Branch	NCI
	D. S. Reiner	Medical Technologist	LPD, NIAID

COOPERATING UNITS (if any) Andrew Plaut, Gastroenterology Div., Tufts New England Medical Center; Peter McCann, Head, Cell Biology Div., Merrill Research; John Bognacki, Sloan-Kettering Research Foundation; Chi-Sun Wang, Oklahoma Medical Research Foundation.

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Parasite Growth and Differentiation Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.50	.75	.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have discovered potent natural secretory defenses in normal human milk (NHM) which are active in vitro against two important intestinal protozoan parasites: Giardia lamblia and Entamoeba histolytica.

1. NHM (from five donors) rapidly killed G. lamblia, but cow's milk and goat's milk did not.
2. The killing was not dependent upon secretory IgA.
3. The parasites were completely protected by bovine or human serum or serum albumin.
4. NHM has two Giardia-cidal activities, one thermolabile and one very heat stable.
5. Entamoeba histolytica and Trichomonas vaginalis were also killed by NHM.
6. It is likely that an unusual lipase is the thermolabile anti-parasitic factor in NHM.
7. The molecular target and mode of Giardia-killing by lipases were explored.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00161-05 LPD

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immunochemistry of Parasitic Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. E. Nash	Senior Investigator	LPD, NIAID
Others:	A. W. Cheever	Assistant Chief	LPD, NIAID
	E. A. Ottesen	Senior Investigator	LPD, NIAID
	F. D. Gillin	Senior Staff Fellow	LPD, NIAID
	L. S. Diamond	Head, PGD Section	LPD, NIAID
	M. N. Lunde	Research Zoologist	LPD, NIAID
	R. Hussain	Guest Worker	LPD, NIAID
	D. B. Keister	Biologist	LPD, NIAID
	N. A. Kaushal	Visiting Fellow	LPD, NIAID
	A. Simpson	Visiting Fellow	LPD, NIAID
	J. Dame	Staff Fellow	LPD, NIAID
	T. McCutchan	Senior Staff Fellow	LPD, NIAID
	M. Hofstetter	Research Associate	LPD, NIAID

COOPERATING UNITS (if any)

C. Tuazon, Guest Worker, LPD, NIAID
P. D. Smith, Guest Worker LMI, NIAID
CDC (Dr. Ruiz-Tiben); Assn. Biol. Chem., LCC, Harvard Medical (Nasir-ud-Din)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.2	PROFESSIONAL: 1.2	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The identification and utilization of specific parasite antigens to study host-parasite interactions continues to be a major focus of this laboratory. The rate of clearance of two specific schistosome excretory-secretory antigens depended upon the nature and composition of the antigen, intensity of infection, the specific antibody response of the host, and size or charge/mass ratio of the specific immune complexes. Secretory membrane components of G. lamblia were studied following radiolabelling of the surface. The major secretory product is a polydisperse hydrophobic material destroyed by proteases and Na periodate. The major secretory product(s) of the two human strains studied differed antigenically and one strain lacked a peptide labelled in the other. In crossed immunoelectrophoresis, the major secretory material was identified as a slightly anodic material present in 3 of the 4 strains tested.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00162-06 LPD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biochemical Cytology of Host-parasite Interactions in Parasitic Protozoa

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. M. Dwyer	Supervisory Microbiologist	LPD, NIAID
Others:	P. R. Gardiner	Visiting Fellow, FIC	LPD, NIAID
	H. W. Sheppard	Staff Fellow	LPD, NIAID
	E. C. Weinbach	Head, Physiology & Biochemistry Section	LPD, NIAID
	T. B. Fioretti	Chemist	LPD, NIAID

COOPERATING UNITS (if any) M. Gottlieb, Dept. Pathobiology, School of Hygiene & Public Health, Johns Hopkins University, Baltimore, MD; E. S. Kaneshiro, Dept. Biological Sciences, Univ. Cinncinati, Cinncinati, OH; M. K. Wassef, NIOSH, CDC, DHHS, Rockville, MD.

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Cell Biology and Immunology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.8	3.8	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Aspects of the cell biology and immunology of host-parasite interactions among several intra- and extracellular parasitic protozoa are being investigated. Leishmania sp. and Trypanosoma sp. are being used as models of intracellular and extracellular parasitism, respectively. Emphasis is placed upon 1) determining some of the basic chemical and antigenic properties of parasite surface membranes; 2) ascertaining the nature and extent of the interactions of parasite surfaces with specific host cell types; 3) defining the basic mechanisms involved in the intracellular survival and multiplication of parasites within host cells; and 4) attempts to determine the means by which parasites circumvent host immuno-defense systems. Techniques employed in these studies include; subcellular fractionation, ultracyto-and immunochemistry, electron microscopy, chromatography, polyacrylamide gel electrophoresis, lectin assays, radio isotope labeling, gel immunoassays, and in vitro cell culture.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00185-03 LPD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Pathogenic Protozoa: Structure, Virulence Factors and Endogenous Viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. F. T. Mattern	Senior Investigator	LPD, NIAID
Others:	W. A. Daniel	Biologist, LPD, NIAID	LPD, NIAID
	P. C. Natovitz	Biologist	LPD, NIAID
	D. B. Keister	Biologist	LPD, NIAID
	L. S. Diamond	Head, Parasite Growth and Development Section	LPD, NIAID

COOPERATING UNITS (if any)
Dr. B. M. Honigberg, University of Massachusetts; Drs. Michael R. Spence, John K. Frost, and Prabodh K. Gupta, The Johns Hopkins University; Dr. Shigeko Nomura, NIC, NIH

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Cell Biology and Immunology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3	PROFESSIONAL: 2	OTHER: 1

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project involves studies on the biology of the parasitic protozoa Trichomonas vaginalis, Entameoba histolytica, and Giardia lamblia. In view of the retirement on June 30, 1982 of the principal investigator, the major emphasis for the nine month period has been on Trichomonas in collaboration with Dr. Michael R. Spence of The Johns Hopkins University School of Medicine involving metronidazole resistant trichomonad strains and therapy for drug resistant trichomoniasis. Current studies which will be continued at The Johns Hopkins University beginning July, 1982 will include the search for properties of trichomonads which may lead to more effective therapy than is currently available for drug resistant trichomoniasis and a screening program to determine the magnitude of the problem of drug resistance in trichomonads.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00197-03 LPD

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immune Recognition in Filariasis and Other Helminth Infections

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Hussain	Senior Staff Fellow	LPD, NIAID
Others:	E. A. Ottesen	Senior Investigator	LPD, NIAID
	N. A. Kaushal	Visiting Fellow	LPD, NIAID
	M. Hofstetter	Research Associate	LPD, NIAID
	H. A. Francis	Medical Staff Fellow	LPD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host Parasite Relations

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The major aim of this project is to characterize immunoglobulin responses in helminth infections (primarily filariasis and schistosomiasis) with emphasis on IgE production, regulation and modulation. Highly sensitive radioimmunoassays have been developed to detect and quantitate both IgG and IgE antibodies. IgE was then determined in various clinical forms of filariasis (chronic pathology, microfilaremia, tropical pulmonary eosinophilia) and schistosomiasis (actue and chronic). In addition, individuals living in endemic areas but without clinical disease are also being simultaneously studied to understand their defense mechanisms. Immediate hypersensitivity (IgE) responses have been found to be modulated by IgG blocking antibody. IgG subclasses are being studied in an attempt to define the carrier of blocking activity. Finally, qualitative characterization of IgE responses in different disease states are being carried out in an attempt to define the allergies to which these responses are directed ("fingerprinting") in order to correlate immune recognition with disease or protective state. This would, in addition, provide information about antigens with better specificity in immunodiagnosis or epidemiologic studies.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00208-02 LPD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

DNA of P. berghei and S. mansoni

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. McCutchan	Staff Fellow	LPD, NIAID
Others:	J. Dame	Staff Fellow	LPD, NIAID
	A. J. G. Simpson	Visiting Fellow	LPD, NIAID
	J. Mullins	Biologist	LPD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Malaria

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.6	2.6	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER
X

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have undertaken projects aimed at characterization of the genome of Plasmodium. Along with the development of polymorphic genetic markers, we have studied the arrangement of particular segments of the genome: (1) genes for stable RNA's, ribosomal RNA, 5S and rRNA (2) DNA that apparently does not code for RNA (3) DNA that codes for defined proteins. We have mapped, by restriction enzyme analysis, the four ribosomal genes of P. berghei . They are not tandemly arranged and in this way, look more like E. coli genes than genes from a eucaryotic organism. The rRNA and 5S genes also appear to be arranged in a manner similar to that found in a procaryotic organism. We have also discovered a segment of the genome whose sequence suggets that it does not code for a protein of any size. Initially, it appears to be highly repeated and regularly dispersed throughout the genome but not in the vicinity of the stable RNA genes. Finally, we are searching for coding regions of the plasmodium genome by using defined cloned probes from other systems as well as developing probes directly from plasmodium.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00240-01 LPD

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Culture Physiology and Antigenic Analysis of Sexual Stages of Malaria Parasites

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Carter	Visiting Scientist	LPD, NIAID
Others:	P. Graves	Visiting Fellow	LPD, NIAID
	D. C. Kaushal	Visiting Fellow	LPD, NIAID
	J. Rener	Staff Fellow	LPD, NIAID
	R. Howard	Visiting Associate	LPD, NIAID
	R. W. Gwadz	Res. Entomologist	LPD, NIAID
	N. Kumar	Visiting Associate	LPD, NIAID
	I. Udeinya	Visiting Fellow	LPD, NIAID
	L. H. Miller	Head, Malaria Section	LPD, NIAID

COOPERATING UNITS (if any) M. Aikawa, Case Western Reserve University, Cleveland, Dyann Wirth, Harvard School of Public Health, Harvard Medical School, Boston

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.65	4.75	1.90

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The mechanism of transmission blocking immunity to Plasmodium gallinaceum in chickens and Aedes aegypti mosquitoes has been examined. Three classes of antibody dependent mechanism one involving complement and two without have been defined. Using radiolabelled preparations of the target cells of this immunity, the gametes, zygotes and ookinetee which occurs in the mosquito midgut, it has been possible to define by immunochemical methods several target antigens.

Antibody independent killing of midgut stages of the parasite by the vertebrate host's alternative pathway of complement has been shown. Parasites survive the complement systems of their natural host by a combination of temporary specific resistance coupled with complement inactivation mediated by the mosquito proteases released into the blood meal.

Considerable diversity in such properties as drug sensitivity and gametocyte production has been demonstrated among clones of P. falciparum. The properties are stable in each clone. Different types of clones representing different combinations of such properties may occur in a single isolate of parasites.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00241-01 LPD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Identification of Receptors for Merozoite Invasion of Erythrocytes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. H. Miller	Head, Malaria Section	LPD, NIAID
Others:	P. David	Rockefeller Foundation, Fellow	LPD, NIAID
	R. Howard	Research Expert	LPD, NIAID
	T. Hadley	Research Associate	LPD, NIAID
	D. Hudson	Biologist	LPD, NIAID
	J. Colligan	Senior Scientist	LI, NIAID
	M. McGinnins	Research Biologist	BB, CC

COOPERATING UNITS (if any) Case Western Reserve University, Cleveland, OH (M. Aikawa)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Malaria

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.2
PROFESSIONAL: 1.2
OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

Erythrocytes

SUMMARY OF WORK (200 words or less - underline keywords)

Invasion of erythrocytes by malarial merozoites requires specific determinants on the surface of erythrocytes to which merozoites attach. Low affinity receptors mediate the initial interaction and apical reorientation; high affinity receptors form an apical junction that precedes entry into the parasitophorous vacuole. Previous studies on Plasmodium knowlesi have shown that the Duffy blood group determinant is involved in junction formation, the high affinity binding. We have now shown that band 3, the major erythrocyte transmembrane protein, is a receptor and is probably involved in the initial recognition. We are now attempting to identify equivalent receptor components on merozoites.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00242-01 LPD

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biological and Biochemical Studies of Antigens on Malaria-infected Red Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. J. Howard	Research Expert	LPD, NIAID
	L. H. Miller	Head, Malaria Section	LPD, NIAID
Others:	S. B. Aley	Guest Worker	LPD, NIAID
	J. W. Barnwell	Staff Fellow	LPD, NIAID
	T. Hadley	Research Associate	LPD, NIAID
	J. H. Leech	Clinical Associate	LPD, NIAID
	T. McCutchan	Sr. Staff Fellow	LPD, NIAID
	I. J. Udeinya	Visiting Fellow	LPD, NIAID
	W. Daniel	Research Associate	LPD, NIAID
	V. Kao	Technician	LPD, NIAID

COOPERATING UNITS (if any) M. Aikawa, Case Western Reserve University; J. Coligan, LI, NIAID; H. Coon, LCB, NCI; I. Green, LI, NIAID; K. Lloyd, Memorial Sloan-Kettering Cancer Center, J. Rener, Hazleton Lab. America, VA; R. Schauer, Christian Albrecht's Universitat; J. Schmidt, LI, NIAID; D. Taylor, Georgetown University.

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.7	5.7	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are characterizing the biological functions and biochemical constitution of new membrane antigens on malaria-infected erythrocytes to ascertain their roles in parasite evasion of host immunity. Plasmodium falciparum-infected erythrocytes express antigenic knobs on their surface which mediate attachment to venular endothelium. Sequestration of infected cells from the peripheral circulation prevents their passage through the spleen. Localized spleen-dependent immune responses are thereby avoided. An in vitro assay for attachment to endothelial cells and melanoma cells has been developed allowing us to explore ways to block attachment. Plasmodium knowlesi infected erythrocytes express a surface variant antigen which changes during chronic infection. We have shown that variant antigen is involved not only in evasion of variant-specific immunity, but also in suppression of other spleen-dependent parasiticidal immune responses. Variant antigen expression per se, and the parasites capacity to alter variant antigen are both dependent on the presence of the host spleen. The variant antigen has also been identified as a membrane protein synthesized by the introarythrocytic malaria parasite.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00243-01 LPD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Characterization of Malarial Proteases: Their Role in Hemoglobin Digestion, Invasion and Rupture.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. J. Hadley	Research Associate	LPD, NIAID
Others:	F. K. Friedman	Staff Fellow	LCB, NIDDK
	A. Schechter	Chief	LCB, NIDDK
	L. H. Miller	Head, Malaria Section	LPD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Malaria

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.6	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES (Blood) ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A comparison of the proteolytic enzymes involved in hemoglobin digestion has been made using a chloroquine (pigmented) sensitive and chloroquine resistant (non-pigmented) strain of Plasmodium berghei. The chloroquine sensitive (pigmented) strain contains a parasite specific enzyme that is found neither in the host cell (erythrocyte or reticulocyte) nor in the chloroquine resistant strain. The chloroquine resistant strain appears not to possess its own enzyme as extracts contain only activities identical to those of the non-infected reticulocyte. Work comparing the chloroquine sensitive and chloroquine resistant Plasmodium falciparum is currently in progress.

Studies on the effects of various protease inhibitors on invasion of the erythrocyte by the malaria parasite indicate that chymostatin at 50 to 100 micromolar concentrations significantly reduces invasion. It appears non-toxic in so far as attachment may occur. Other inhibitors including antipain, PMSF, and Pepstatin have no effect. The data suggests that proteolytic enzymes may play a role in invasion. Studies are underway to identify and characterize these enzymes.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00244-01 LPD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Developmental Adaptations of Trypanosoma cruzi to the Vertebrate Immune System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Sher	Head, Cell Biology and Immunology Section	LPD, NIAID
Others:	L. V. Kirchhoff	Medical Staff Fellow	LCI, NIAID

COOPERATING UNITS (if any)
Wellcome Research Laboratories, Kent, England (Dr. D. Snary)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Cell Biology and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.8	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this project we have been examining developmental adaptations of Trypanosoma cruzi to the vertebrate immune system and, in particular, the transformation of complement sensitive epimastigotes into complement resistant trypomastigotes. An in vitro system has been developed for studying this transformation and a membrane determinant identified by means of monoclonal antibodies which regulates this developmental change.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00245-01 LPD

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Macrophage Involvement in Immunity to Schistosoma mansoni Infection

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. L. James	Guest Worker	LPD, NIAID
Others:	A. Sher	Head, Cell Biology and Immunology	LPD, NIAID
	M. Meltzer	Senior Investigator	LIB, NCI
	J. Lazdins	IPA	LIB, NCI
	R. Oliveira	Guest Worker	LPD, NIAID

COOPERATING UNITS (if any)

Biomed Research Institute, Rockville, MD (Dr. F. Lewis)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Cell Biology and Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.6	PROFESSIONAL: 1.5	OTHER: .1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The goal of this project is to evaluate the involvement of activated macrophages macrophages as effector cells of immunity to infection by the helminth parasite, Schistosoma mansoni. Three mouse models of resistance are being explored in this study: (1)"concomitant immunity" i.e., the partial resistance to challenge S. mansoni infection that develops as a consequence of ongoing primary infection, (2) BCG-induced resistance, and (3) vaccination with highly irradiated S. mansoni cercariae. In each of these models macrophages activated for in vitro killing of extracellular targets (tumor cells, skin-stage schistosomula) can be elicited. Production of macrophage activating factors after specific antigen challenge of S. mansoni-infected or vaccinated mice in vivo or of lymphoid cells from these mice in vitro has been observed. When development of S. mansoni-induced (concomitant immunity model) was examined in several inbred strains of mice previously characterized as displaying defective macrophage activation for tumor cytotoxicity after BCG infection in vivo, a correlation was observed between macrophage activity and resistance to challenge infection. These studies suggest that activated macrophages may play a role in immunity to this parasite.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00246-01 LPD

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Molecular Studies of the Genome and Surface of Schistosoma mansoni

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. J. G. Simpson	Visiting Fellow	LPD, NIAID
Others:	T. F. McCutchan	Senior Staff Fellow	LPD, NIAID
	D. Singer	Senior Staff Fellow	I, NCI
	T. E. Nash	Medical Officer	LPD, NIAID
	R. Correa-Oliveira	Guest Worker	LPD, NIAID
	S. James	Guest Worker	LPD, NIAID
	A. Sher	Head, Cell Biology and Immunology Section	LPD, NIAID

COOPERATING UNITS (if any)

Biomedical Research Institute, Rockville, MD (F. Lewis & C. Richards)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Cell Biology and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The rRNA gene from Schistosoma mansoni has been cloned. The gene is approximately 10K bases in length and is repeated 500-1,000 times. Most copies are identical but divergent copies of the gene have been identified. Different schistosome strains and species can be distinguished by differences in these genes. The surface of the schistosome, represents a prime target for immune attack. Serum from mice vaccinated with highly irradiated cercariae has been utilized to immunoprecipitate surface labelled polypeptides. A dominant species with a molecular weight of approximately 32,000 has thus been identified. Lectin probes have identified glucose and/or mannose, galactose and N-acetylglucosamine as dominant monosaccharides on the schistosomula surface. Maturation of the parasite in vivo is concomitant with a decrease in the density of these exposed carbohydrate moieties and an increase in the amount of detectable sialic acid. Monoclonal antibodies have been utilized to probe the nature of the H-2 determinants present on the parasite surface and indicate the presence of intact glycoprotein antigens. The absence of H-2 like genes in the parasite genome suggests that the antigens are acquired from the host and not synthesized by the parasite.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00247-01 LPD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immunochemical Analysis of Cloned Trypanosoma cruzi Stocks

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	V. Bongertz	Visiting Fellow	LPD, NIAID
Other:	J. A. Dvorak	Res. Microbiologist	LPD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Physiology and Biochemistry

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The antigenic constitution of 2 strains and 6 cloned Trypanosoma cruzi stocks is being analysed immunoelectrophoretically. Five to 7 of the antigens detected were common to all stocks tested. However, 1 to 2 stock-specific antigens were also found, demonstrating interclonal antigenic differences within a T. cruzi strain, i.e., that the population of organisms which constitute a T. cruzi strain is antigenically heterogenous.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00248-01 LPD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Physiology and Genetics of Anopheles Mosquitoes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. H. Collins	Staff Fellow	LPD, NIAID
Others:	R. W. Gwadz	Research Entomologist	LPD, NIAID
	P. Graves	Visiting Fellow	LPD, NIAID
	L. C. Koontz	Biologist	LPD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Malaria Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.9	PROFESSIONAL: 1.0	OTHER: 0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Work has focused on the interaction between the primate malaria Plasmodium cynomolgi and the mosquito Anopheles gambiae, with special attention to factors which cause the normal process of mosquito-parasite infection to fail. Two lines of An. gambiae have been selected which are (respectively) refractory and susceptible to infection with P. cynomolgi. Although the lines are not yet pure, preliminary crosses suggest that susceptibility is dominant and inheritance may be monofactorial. Examination of the susceptibility of these two lines to other species of malaria has revealed the same refractoriness/susceptibility pattern with P. knowlesi and P. berghei. Although both lines are susceptible to infection with the human malarias P. vivax and P. falciparum, the refractory line does manifest consistently reduced susceptibility when compared with the non-selected and susceptible lines.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00251-01 LPD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies on the Immunologic Basis of Resistance in Mice Vaccinated with Irradiated Cercariae

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Sher	Head, Cell Biology and Immunology Section	LPD, NIAID
Others:	S. L. James	Guest Worker	LPD, NIAID
	R. Oliveira	Guest Worker	LPD, NIAID
	R. Asofsky	Chief	LMI, NIAID

COOPERATING UNITS (if any) Biomedical Research Institute, Rockville, Maryland (Drs. F. Lewis and M. Stirewalt).

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Cell Biology and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.3	PROFESSIONAL: 1.6	OTHER: 0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mice vaccinated with irradiated cercariae of S. mansoni develop resistance to challenge infection in the absence of overt pathology. In order to identify mechanisms of parasite rejection in this model, mice with genetic or induced immunodeficiencies have been analyzed and compared for their vaccine induced resistance and schistosome specific immune responses. The results of these studies suggest that in the mouse, vaccine induced immunity is dependent on both B and T lymphocytes and that the effector mechanism of parasite rejection in this model may involve both a humoral and cellular component. Neither complement nor mast cells appear to play a role in this effector mechanism. A strain survey has revealed a polymorphism in the ability of different inbred mice to respond to the vaccine and genetic experiments have indicated that the expression of high levels of immunity is inherited as a dominant trait. The genes involved in the control of resistance also effect the levels of antibody produced in response to the vaccine. One of these genes is linked to the mouse major histocompatibility.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00252-01 LPD

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cell Biological Studies of Trypanosoma Cruzi Clones Isolated from Chronic Chagasic Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Juan C. Engel	WHO Fellow	LPD, NIAID
Others:	James A. Dvorak	Res. Microbiologist	LPD, NIAID
	Patricia Engel	Guest Worker	LPD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.6	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Single-cell-isolated Trypanosoma cruzi clones were obtained from Triatoma infestans, fed on either a chronic chagasic patient (ten clones) or an animal infected with T. cruzi strain CA-I (nine clones). The growth kinetics in liquid medium (LIT) and peak Coulter volume of these clones were characterized. Significant inter- and intra-group differences in growth rates and peak modal volumes were found. These data indicate that genetically heterogeneous subpopulations of T. cruzi exist in chronic chagasic patients and in T. cruzi strains.

A simple protocol was developed for serial in vitro cultivation of the intracellular amastigote form of T. cruzi in cell-free medium (LIT). The amastigotes are resistant to complement-mediated lysis. In unperturbed cultures, amastigotes grow for two cycles and differentiate to epimastigotes which begin a second exponential growth phase. Continuous passage of growth-phase amastigotes to fresh medium inhibits transformation to epimastigotes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER
		Z01 AI 00253-01 LPD

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies of the Immunologic Responses to Filarial Infections

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. A. Ottesen	Senior Investigator	LPD, NIAID
Others:	R. Hussain	Senior Staff Fellow	LPD, NIAID
	N. Kaushal	Visiting Fellow	LPD, NIAID
	H. Francis	Medical Staff Fellow	LPD, NIAID
	M. N. Lunde	Research Zoologist	LPD, NIAID
	T. J. Lawley	Medical Investigator	DB, NCI

COOPERATING UNITS (if any)

Tuberculosis Research Center, Madras, India (Dr. S. P. Tripathy); Medical College of Madras, India (Prof. K. V. Thiruvengadan); Johns Hopkins Medical Center (Dr. R. G. Hamilton).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host Parasite Relations

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.11	1.5	.6

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

IgE antibodies are likely important in protective immunity to filaria and other helminths. Techniques have been developed to define these antibodies both quantitatively and qualitatively. Patients with the tropical eosophilia syndrome have enormous elevations of specific anti-filarial IgE antibody, while those with microfilaremia have the low levels, a finding apparently reflecting not qualitative differences in allergen recognition but differences in immunoregulation of IgE antibody production. All patients have sufficient IgE to mediate allergic reactions to the parasite but paradoxically almost none have such reactions. IgG "blocking antibodies" appear to develop as a primary modulating mechanism of allergic responsiveness in these patients. The subclass restriction of these IgG antibodies is being defined.

Because present clinical, parasitologic- or serologic methods are inadequate to diagnose many patients with filariasis, studies have focused on evaluating cross reactivity among helminth parasites and identifying with a variety of immunochemical techniques unique antigens that are parasite stage or species-specific. The findings have been utilized to develop other assays capable of detecting parasite antigen in the sera of infected individuals.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00254-01 LPD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies of the Host Response in Onchocerciasis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. A. Ottesen	Senior Investigator	LPD, NIAID
Others:	H. Francis	Medical Staff Fellow	LPD, NIAID
	R. Hussain	Senior Staff Fellow	LPD, NIAID
	N. A. Kaushal	Visiting Fellow	LPD, NIAID
	M. M. Frank	Chief	LCI, NIAID
	M. Kaliner	Head, Allergy Section	LCI, NIAID
	T. J. Lawley	Medical Investigator	DB, NCI

COOPERATING UNITS (if any) Onchocerciasis Chemotherapeutic Res. Ctr., Tamale, Ghana (Dr. K. Awadzi); Dept. of Med., Mayo Clinic, Rochester, MN (Drs. G.J. Gleich & S.K. Ackerman); Dept. of Med., SUNY, Stoneybrook, NY (Dr. A.P. Kaplan); Special Programme for Tropical Disease Res., WHO, Geneva, Switzerland

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Host Parasite Relations

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this project is to study the host response to onchoreceal infection in order to understand the pathogenesis of clinical disease, the immune mechanisms operative in the persistence of the parasite within the host and in protective immunity, and to develop improved immunodiagnostic techniques.

The initial phase of this project has involved a detailed clinical and laboratory assessment of the severe side effects (Mazzotti reaction) that accompany treatment of the infection and limit the potential of mass chemotherapy for onchocerciasis. Twenty-five patients were treated at a research center in Ghana and subjected to intensive immunologic and clinical evaluation as their prolonged Mazzotti reactions developed over the first week of therapy. The results from the study are currently being accumulated.

Evaluation of IgE and IgG antibodies from patients with onchocerciasis has suggested that there is greater species specificity (i.e. less cross-reactivity), and therefore more potential for practical immunodiagnosis, in the IgE antibody system. The antigens and allergens underlying these specific responses are now being identified and isolated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00255-01 LPD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Studies of the Immunologic Responses to Schistosome Infections

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. A. Ottesen	Senior Investigator	LPD, NIAID
Others:	M. Hofstetter	Research Associate	LPD, NIAID
	R. Hussain	Senior Staff Fellow	LPD, NIAID
	M. Barral-Netto	Visiting Fellow	LPD, NIAID
	A. W. Cheever	Assistant Chief	LPD, NIAID
	T. E. Nash	Senior Investigator	LPD, NIAID
	T. J. Lawley	Medical Investigator	DB, NCI

COOPERATING UNITS (if any)
San Juan Laboratories, Center for Disease Control (Dr. E. Ruiz-Tib and Dr. R. A. Hiatt)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Host Parasite Relations

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.10	1.4	0.6

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Clinical studies have defined a form of limited immunosuppression, in patients with chronic schistosomiasis (with S. mansoni or S. mekongi that develops progressively after initial infection. This immune suppression is characterized by poor or absent lymphocyte to proliferative responsiveness to parasite antigens in vitro and diminished immediate hypersensitivity (IH) responses to parasite antigen in vivo. The lymphocyte suppression is effected by various serum factors, by adherent mononuclear cells and by histamine released from the patients' IgE-coated basophils. IH responses are modulated by IgG 'blocking antibodies' directed against the same immunogens that evade IgE antibody responses. Long-term follow-up of treated patients indicates that these suppressive mechanisms diminish after therapy.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00256-01 LPD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Regulation of Immunity to Pathogenic Protozoa

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. L. Sacks	Staff Fellow	LPD, NIAID
	P. A. Scott	Staff Fellow	LPD, NIAID
	A. Sher	Head, Cell Biology and Immunology Section	LPD, NIAID
Others:	R. Asofsky	Chief	LMI, NIAID
	F. A. Neva	Chief	LPD, NIAID

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Cell Biology and Immunology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) A. Anti-idiotypic antibodies have been used to immunize mice against infection with African trypanosomes. Administration of anti-idiotypic antibodies prior to infection appear to prime for the expression of idiotype bearing, parasite specific clones. This is the first demonstration that anti-idiotypic antibodies can substitute for antigen in the induction of anti-microbial immunity. B. The role of antibody in the susceptibility of mice to Leishmania infection was studied in μ-suppressed B cell depleted BALB/c mice. Unexpectedly, these mice displayed dramatically enhanced resistance to infection with L. tropica and L. mexicana, suggesting that the high antibody titers which accompany chronic leishmaniasis may play a regulatory role. C. The factors controlling macrophage mediated killing of different Leishmanias were studied. Strains of Leishmania from patients with chronic muco-cutaneous leishmanasis were relatively resistant to killing when compared with healing strains. In addition, temperature significantly influenced macrophage mediated killing and may be responsible for the chronicity of certain forms of cutaneous leishmaniasis. To further study these phenomena, the parameters of lymphokine production by lymphocytes from Leishmania killing has been developed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00257-01 LPD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immunology of Strongyloidiasis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. A. Neva	Chief	LPD, NIAID
Others:	A. Gam	Bio. Lab. Tech.	LPD, NIAID
	J. Harper	Res. Veternarian	EP, NINCDS

COOPERATING UNITS (if any) Hahnemann Med. School, Philadephia, PA (R. Genta)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Cell Biology and Immunology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.4	PROFESSIONAL: .9	OTHER: .7

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The experience with the ELISA test for detection of antibodies to Strongyloides stercoralis continues to increase. The antigen is an aqueous supernate extracted from filariform(L3) larvae, grown in fecal cultures. The parasite is obtained from patients encountered at the NIH, or from Patas monkeys infected with the human parasite. About 90% of parasitologically proven cases have antibody at a 1:8 serum dilution or greater. The same antigen, as well as a secretion-excretion(metabolic) antigen from the L3's, also stimulate histamine release when exposed to peripheral basophils from infected individuals. The Patas monkey has been found to be susceptible to infection with human S. stercoralis. The Patas monkey appears to be a model for the hyper-infection syndrome, a sometime fatal complication of steroid treatment or other immuno-suppression of human infection with this parasite.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00258-01 LPD

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Development of Stable, Reproducible Models for Chagas' Disease using Trypanosoma cruzi Clones and Inbred Mouse Strains

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Miriam Postan	WHO Fellow	LPD, NIAID
Others:	James A. Dvorak	Res. Microbiologist	LPD, NIAID

COOPERATING UNITS (if any)

National Heart, Lung and Blood Institute (Patterson, R. E. and Palmeri, S.)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

X

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The study is concerned with the course of infection in two inbred mouse strains of two T. cruzi clones and a strain isolated from an acute human case of American Trypanosomiasis. Through the use of cloned stocks the existence of intrastrain subpopulations differing in their pathogenicity for an inbred strain was intermediate between that of the two clones. The inbred mouse-T. cruzi clone is a highly stable and reproducible experimental system.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00093-06 LPD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies of the Immunologic Responses to Helminth Infections

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: E. A. Ottesen

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project terminated October 1, 1981, replaced with project numbers Z01 AI 00253-01, Z01 AI 00254-01, and Z01 AI 00255-01

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00149-06 LPD

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Physiology of Anopheles Mosquitoes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: R. Rosenberg

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project terminated October 1, 1981.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00174-04 LPD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Culture, Physiology and Antigenic Analysis of Sexual and Asexual Erythrocytic Malaria Parasites.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: R. Carter
R. Howard
L. Miller

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project terminated October 1, 1981, replaced with project numbers Z01 AI 00240-01, Z01 AI 00241-01, and Z01 AI 00242-01.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00198-02 LPD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Host Immunological Response to Giardia lamblia Infection

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT
PI: P. Smith

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project terminated October 1, 1981

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00207-01 LPD

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mechanisms of Immunity and Immune Evasion in Parasitic Infection.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: A. Sher

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project terminated October 1, 1981, replaced with project numbers Z01 AI 00244-01 and Z01 AI 00251-01.

PHS-6040
(Rev. 2-81)

LABORATORY OF VIRAL DISEASES
1982 Annual Report
Table of Contents

Z01 AI Project Number		Page
Summary		26-1
00011-17	Studies of Small DNA Containing Viruses Belonging to the Family Parvoviridae. -- Hoggan	26-4
00019-07	Studies on the Treatment of Disease with the Interferon System. -- Levy	26-5
00280-01	Genomic Organization of Ecotropic Murine Leukemia Virus Proviral Sequences in Inbred Mice. -- Buckler	26-6
00281-01	Molecular Cloning of Endogenous MuLV Proviral DNA Sequences from AKR/J and BALB/c DNA. -- Buckler	26-7
00282-01	Malignant Lymphomas Occurring in Congenic Mice. -- Fredrickson	26-8
00283-01	The Pathogenesis of Chronic Myelogenous Leukemia in NFS/N Mice. -- Fredrickson	26-9
00284-01	Characterization of Pathogenic Murine Leukemia Viruses. -- Hartley	26-10
00285-01	Studies of Factors Regulating Xenotropic Murine Leukemia Virus Expression. -- Hartley	26-11
00286-01	Tissue Culture Studies of Genetic Resistance to Murine Leukemia Viruses. -- Hartley	26-12
00287-01	Chromosome Localization of Endogenous Xenotropic MuLV Proviral DNA Sequences in Inbred Mice. -- Hoggan	26-13
00288-01	Molecular Cloning of Endogenous Xenotropic MuLV Proviral DNA. -- Hoggan	26-14
00289-01	Organization of Endogenous Proviral DNAs of Xenotropic Murine Leukemia Viruses. -- Hoggan	26-15
00299-01	Genetic Control and Mapping of Endogenous Proviruses. -- Kozak	26-16
00300-01	Genetic Control of Resistance to Friend Virus in Wild Mouse Populations. -- Kozak	26-17
00301-01	Somatic Cell Genetic Studies on Endogenous Proviruses. -- Kozak	26-18

00302-01 Congenic Mouse Strains Carrying Genes of Importance to Murine Leukemia Virus Infection. -- Rowe 26-19

00303-01 *In vivo* Studies of Genetic Resistance to Murine Leukemia Viruses and Virus-Induced Neoplasms. -- Rowe 26-20

00304-01 Hematologic and Genetic Studies of Malignancies Induced by Friend Helper Virus (F-MuLV). -- Silver 26-21

00305-01 Studies on the Mechanism of *Fv-1*^{b} Inhibition of AKR Thymomagenesis. -- Silver 26-22

00306-01 Studies on the Biology of Mousepox and Development of a Specific Serologic Test for Ectromelia virus. -- Wallace 26-23

PHS-NIH
SUMMARY REPORT

ANNUAL REPORT OF THE LABORATORY OF VIRAL DISEASES, NIAID
October 1, 1981 to September 30, 1982

Dr. Wallace P. Rowe
Chief, Laboratory of Viral Diseases

The Laboratory of Viral Diseases has undergone several significant changes in the past year, with regard to both personnel and program.

Two scientific groups have been transferred into LVD. Dr. Herbert Morse's group, which has been collaborating closely with LVD scientists for a number of years, has become part of LVD, both physically and administratively; this group will provide an extremely important area of expertise for the studies of endogenous retrovirus expression and of cellular factors involved in viral leukemogenesis. Also, Dr. Gordon Wallace's program on the epidemiology and diagnosis of ectromelia virus infection has been placed under LVD. This is a short term program involving the high containment system of Building 41.

With regard to program, the great majority of the work is still focussed on murine retroviruses, with increasing emphasis on the biochemical analysis of endogenous genomes and the genetics of susceptibility to viral leukemias. We are also fortunate to be involved in a collaborative study on the viral genetics of leukemogenicity, with Dr. Nancy Hopkins of MIT; this study now involves *in vitro* constructed recombinant viruses from molecularly cloned parental genomes.

During the past year Dr. Padman Sarma, NCI, has been a guest worker in LVD, and has developed a sensitive neutralization test for spontaneous antibody to endogenous mouse retroviruses. This test should be of great value to LVD in our studies of factors affecting retrovirus expression and leukemogenicity.

Rmcf gene a major determinant of two forms of viral leukemia. In tests of F1 hybrids and segregating crosses it has become clear that the *Rmcf* gene, detected initially by its inhibition of MCF virus infectivity in tissue culture, inhibits both thymic lymphomagenesis and Friend helper virus erythroblastic leukemias. (Rowe, Hartley).

H-2-linked regulation of xenotropic virus expression. Thymus cells of F/St mice express high levels of X-MuLV but this phenotype is markedly suppressed in most F1 hybrids with other inbred mouse strains. A single gene governing this suppression has been identified; called *Cxv-1*, it has been mapped to chromosome 17, very close to the *H-2^k* locus. (Hartley, Morse, Yetter).

Leukemogenicity of *in vitro* recombinant viruses. Recombinant viruses derived by splicing pieces of ecotropic and MCF MuLV cloned viral DNAs are being tested for leukemogenicity and other biological properties. Two viruses in which only the LTR or LTR plus p15E segments come from the leukemogenic MCF progenitor have been found to accelerate thymic lymphoma in AKR mice. (Hartley, Rowe, Hopkins (MIT)).

Establishment of strains of mice congenic for specific MCF virus resistance gene. The resistant allele of *RMcf*, located on chromosome 5 near the *Hm* locus, is being bred onto the NFS mouse background from both DBA/2 and CBA/N. Segregating populations are being tested for sensitivity to spontaneous, carcinogen, and virus-induced neoplasia. (Hartley, Rowe).

A novel cell surface receptor is required for MCF viral replication. Since Chinese hamster cells do not efficiently support the replication of ecotropic, xenotropic, amphotropic or MCF MuLVs, hamster/mouse somatic cell hybrids are being used to identify, chromosomally map and isolate receptor loci for these viruses. Results show that a novel receptor is required for MCF viral replication and that neither ecotropic nor xenotropic MuLVs replicate in cells with the MCF receptor. (Kozak).

Differential expression of endogenous C-type viral genes isolated in somatic cell hybrids. The expression of infectious virus induced by 5-iododeoxyuride (IUdR) or 5-azacytidine has been examined in somatic cell hybrids bearing specific V loci with few, if any, other mouse chromosomes. Results show clearly different kinetics of expression for Bxv-1 (rapid induction and turn off) and Akv-2 (continued expression). Thus, the kinetics of virus expression is determined by these proviral loci or by cis-acting regulators (Kozak).

Locus for xenotropic virus carried on chromosome 1 in many mouse strains. In 7 inbred strains, a xenotropic virus-inducing locus has been located on chromosome 1. In 6 of these strains the sites are allelic (Bxv-1); the chromosome 1 locus of F/St may not be allelic with Bxv-1. Neither of the 2 loci carried by NZB mice maps at or near Bxv-1 and an additional unlinked locus. The Asian mouse, M. m. molossinus carries Bxv-1 and at least 2 additional xenotropic virus inducing loci. (Kozak, Morse).

Genetic control of resistance to Friend virus in wild mouse populations. The Japanese mouse, Mus musculus molossinus, carries a locus apparently identical to the Friend virus resistance gene, Fv-4, described originally in G strain mice. Studies on hybrid mice have resulted in the identification of a second locus on chromosome 5 which influences sensitivity to Friend virus, and the description of a sexual bias in susceptibility to disease. Genetic crosses are currently being tested to determine whether any of the novel MuLV reactive fragments identified by blot hybridization in Fv-4^r congenics are associated with the resistance allele. (Kozak).

Genetic mapping of endogenous proviruses. The chromosomal locations of 9 V loci for the expression of both eco and xeno C-type viruses have now been established. Recent data show that the CBA/J ecotropic locus is allelic with that of BALB/c. A provirus for the B-type mammary tumor virus was mapped to chromosome 1 in 3 mouse strains (NS, DBA, GR) using a combination of blot hybridization and sexual genetics. (Kozak, Rowe, V. Morris).

Chromosomal distribution of xenotropic proviral DNA. The molecularly cloned xenotropic MuLV specific fragment has been used to analyze somatic cell hybrids. Viral sequences are scattered on most if not all mouse chromosomes. A hybrid carrying the isolated distal fragment of chromosome 1 bearing the induction locus Bxv-1 was shown to carry 3 fragments with viral sequences. Restriction enzyme analysis identified one as containing a typical xenotropic envelope and one as having an MCF type env region. (Hoggan, Kozak).

Germline reinsertions of MuLV detected biochemically. The examination of DNAs isolated from sublines of AKR mice by Southern-blot analysis suggests that a continuing, subline independent acquisition of new inserts of ecotropic murine leukemia proviral copies is occurring in the germline DNA of these high virus expression mice. Ecotropic proviral DNA copies shared by the four AKR sublines studied are related to their geneology. AKR thymoma DNA contains additional copies of ecotropic MuLV proviral sequences unique to each tumor. RNAs isolated from spontaneous AKR thymomas contain, in addition to the 35S and 21S viral specific mRNAs, additional RNA sequences that hybridize to various DNA probes derived from subgenomic regions of MuLV proviruses. (Buckler, Rowe).

Cloning of endogenous retroviral genomes. The molecular cloning of restriction fragments from normal mouse liver DNA and AKR thymoma DNA that contain endogenous MuLV proviral sequences and their flanking cellular sequences is in progress. These clones will be used to assess the role of adjacent mouse cellular DNA sequences in the expression of MuLV virion and mRNAs and the role of MuLV sequences in the establishment and maintenance of thymomas in AKR mice. (Buckler, Martin).

Mouse genes affect target cell for transformation by Friend MuLV. Friend helper virus causes erythroleukemia in BALB/c mice but lymphoid and myelogenous leukemia in C57BL/6 mice. Studies with backcross, congenic and recombinant inbred strains suggest that this trait is controlled by one or two mouse genes distinct from previously identified Friend virus loci. (Silver).

Fv-1^b restriction of in vivo is mediated by thymocytes not thymic stroma. Introduction of the Fv-1^b allele into AKR mice reduces the incidence of thymic lymphoma as well as the titer of endogenous ecotropic virus in the thymus. Studies with thymic chimeras suggest that the virus phenotype is controlled by the Fv-1 type of thymocytes rather than the Fv-1 type of the thymic stroma. (Silver).

B cell lymphomas associated with murine C-type viruses. NFS Swiss mice congenic for ecotropic V loci from AKR and C58 have a fairly high incidence of spontaneous lymphomas. The majority of lymphomas originate from B lymphocytes rather than T as in AKR and C58. This host dependent shift in neoplastic effect has allowed an extensive study on various aspects of B cell lymphomas including cell surface antigens, development of transplantable B lymphoma lines, as well as detailed histologic and cytologic characteristics of lymphomas at different stages of development. (Fredrickson, Morse, Hartley).

Ecotropic virus regularly induces myelogenous leukemia. An ecotropic virus originally isolated from a wild mouse induces with few exceptions chronic myelogenous leukemia. Extreme proliferation of the myelogenous precursor, concomitant with clinical leukemia, indicates that the neoplastic effect of this virus is restricted to a single target, the CFU-GM. This selectivity is unique among murine retroviruses. (Fredrickson, Hartley).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00011-17 LVD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies of small DNA containing viruses belonging to the family Parvoviridae.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. David Hoggan, Sc.D.	Staff Scientist, LVD, NIAID

COOPERATING UNITS (if any) K.I. Berns, Univ. Florida, College of Medicine, Gainesville, Florida; F. Brent Johnson, Depart. Microbiology, Brigham Young University, Provo, Utah.

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Viral Oncology Section

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.05	.05	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The PI has a continuing interest in the area of parvovirus research but other priorities have limited his participation to consultation and providing reagents (seed stocks antisera and cell clones) during the past year. He will continue these activities at the present low level at little or no cost to the Institute.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00019-07 LVD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

STUDIES ON THE TREATMENT OF DISEASE WITH THE INTERFERON SYSTEM

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Hilton B. Levy	Head, Molecular Virology Section	LVD	NIAID
Other:	Freddie Riley	Chemist, Molecular Virology Section	LVD	NIAID

COOPERATING UNITS (if any) G. Baer, CDC; A. Salazar, Walter Reed; D. MacFarlin, NINCDS; A. Levine, NCI; R. Herberman, NCI; M. Chirigos, NCI; B. Lampkin, Childrens Cancer Testing Group; S. Hortobagyi, M.D. Anderson Hospital; G. Rakela, USC; K. Engle, U.S. Cal.; B. Leventhal, Johns Hopkins Hospital, R. Oldham, NCI; J. Reed, Portsmouth Naval Hospital

LAB/BRANCH
Viral Diseases

SECTION
Molecular Virology

INSTITUTE AND LOCATION
NIAID

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1	.5

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☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Five new clinical programs, treating cancer and virus diseases with poly ICLC have been approved and patients enrolled. Experience obtained from previous studies have enabled us to decrease toxic manifestations, and still maintain good levels of interferon. Encouraging clinical results continue to be seen with the neurologic paralytic disease associated iwth immune dysfunction and juvenile larymyopopilloma, but these are not controlled studies. No statement can be made about therapeutic value in the other clinical studies as yet. In a phase I study, giving the drug i.m., it was shown that levels of drug that are insufficient to induce detectable levels of interferon, still could augment N.K. cell activity in man, while exogenous interferon administration was inhibitory to N.K. cell activity. In mice, poly ICLC increases the number of pluripotential stem cell colonies in irradiated mice, increases colony stimulating factor, and activates macrophages.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00280-01 LVD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Genomic organization of ecotropic murine leukemia virus proviral sequences in inbred mice.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles E. Buckler	LVD, NIAID
Other:	M. David Hoggan, Sc.D.	LVD, NIAID

COOPERATING UNITS (if any)
M.A. Martin (LMM, NIAID); S.P. Staal (Department of Oncology, The Johns Hopkins Univ.)

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SECTION
Viral Oncology Section

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, Bethesda, Maryland

TOTAL MANYEARS: 2/3	PROFESSIONAL: 2/3	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Individual DNAs isolated from four sublines of AKR mice (AKR/J, AKR/N, AKR/Cum, and AKR/Boy) have been examined by Southern-blot analysis following digestion with several restriction enzymes. A 500 base pair, ^{32}P-labeled, cloned ecotropic murine leukemia virus (MuLV) envelope-specific probe was used to identify restriction fragments that contain ecotropic MuLV proviral sequences. Variation in the number of proviral copies and in their genomic organization as reflected by the location of restriction enzyme sites in flanking cellular sequences was observed both between and within AKR sublines. The results obtained are consistent with a continual acquisition of new proviral copies in the four sublines studied. Ecotropic proviral DNA copies shared by the four AKR sublines are related to their geneology; each subline contains two or three copies of proviral DNA in common with other sublines and from one to six unique ecotropic proviruses. Some of the unique proviral DNA copies contain internal alterations as reflected by restriction enzyme maps that differ from those of prototype ecotropic provirus.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00281-01 LVD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Molecular cloning of endogenous MuLV proviral DNA sequences from AKR/J and BALB/c DNA.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Charles E. Buckler LVD, NIAID

COOPERATING UNITS (if any)

T. Theodore and M. A. Martin (LMM, NIAID)

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Viral Oncology Section

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1/3	1/3	

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The molecular cloning of restriction fragments isolated from mouse DNA that contain sequences that hybridize to an ecotropic MuLV specific probe is in progress. Preliminary analysis by Southern blot hybridization determined the size in kilobases of the fragments of interest. Digested DNA was then fractionated by size by preparative agarose gel electrophoresis. The fragments containing ecotropic proviral sequences were isolated and are being cloned in cosmid vectors. These clones will be used in studies to determine if the basis for the difference in ecotropic MuLV expression in AKR and BALB/c mice results from differences in the proviral sequences or in the flanking cellular sequences. A similar approach will be employed in the isolation and molecular cloning of unique DNA restriction fragments that have been observed in AKR/J thymoma DNAs. The role of new MuLV inserts the generation of thymoma will be examined by DNA and RNA blot hybridization studies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00282-01 LVD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Malignant lymphomas occurring in congenic mice.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Torgny N. Fredrickson, D.V.M., Ph.D.	(LVD, NIAID)
Others:	Wallace P. Rowe, M.D.	(LVD, NIAID)
	Janet W. Hartley, Ph.D.	(LVD, NIAID)
	Christine Kozak, Ph.D.	(LVD, NIAID)
	Herbert C. Morse, III, M.D.	(LVD, NIAID)
	Wendy Davidson, Ph.D.	(LVD, NIAID)
	Robert Yetter, Ph.D.	(LVD, NIAID)

COOPERATING UNITS (if any)

Dr. P. Pattengale (Univ. Southern California)

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SECTION
Viral Oncology Section

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases

TOTAL MANYEARS: 4/12	PROFESSIONAL:	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Characterization of lymphomas occurring in old mice has been carried out by studying the histopathology, karyology, cell surface antigens, intracytoplasmic immunoglobulin light and heavy chains, development of cell lines, both in vivo and in vitro. Identification of these lymphomas to be composed of B lymphocytes, mainly large and/or small follicular center cells, has been established. In addition, 4 cases of chronic lymphocytic leukemia and 2 of pre-B lymphoma have been identified. All cell lines developed in vitro (6) are of B lymphocytes as well as those studied for surface antigens of the 9 passaged in vivo. The in vitro lines have generally 41 chromosomes and the extra chromosome is being identified. Thus, as in humans, lymphomas associated with advanced age in mice are derived from clones (intracytoplasmic Ig substantiates the clonal origin) of malignant B lymphocytes, most of which are in a fairly advanced stage of differentiation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00283-01 LVD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
The pathogenesis of chronic myelogenous leukemia in NFS/N mice.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Torgny N. Fredrickson, D.V.M., Ph.D.	(LVD, NIAID)
Others:	Wallace P. Rowe, M.D.	(LVD, NIAID)
	Janet W. Hartley, Ph.D.	(LVD, NIAID)

COOPERATING UNITS (if any)

Drs. T. MacVittie and M Padgett (Armed Forces Radiobiology Institute)

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Viral Oncology Section

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, Bethesda, Maryland

TOTAL MANYEARS: 1/12	PROFESSIONAL:	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A retrovirus isolated originally from a California wild mouse, induces chronic myelogenous leukemia. This unique virus provided an opportunity to follow the development of this disease during the preleukemic (11 mice) and leukemic phases (14 mice). Stem cells, including CFU-E, BFU-E, CFU-S, and CFU-GM were enumerated and the stage of disease evaluated by differential counts of marrow and spleen cells, spleen weight and histology. During the preleukemic phase no severe alterations occurred for any aspect measured. Six months after inoculation the number of CFU-GM increased in both the marrow and spleen; CFU-E and BFU-E decreased in the marrow but increased moderately in the spleen. CFU-S could not be measured in the leukemic mice but were moderately elevated in preleukemics. The result of this study, indicating a lack of a distinguishable preleukemic phase, is being reevaluated in an ongoing second study.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00284-01 LVD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Characterization of pathogenic murine leukemia viruses.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Janet W. Hartley, Ph.D.	LVD, NIAID
Other:	Wallace P. Rowe, M.D.	LVD, NIAID
	Jonathan Silver, M.D.	LVD, NIAID

COOPERATING UNITS (if any)
Nancy Hopkins (MIT); Miles W. Cloyd (NIAID, RML)

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Viral Oncology Section

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	.25	1.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The virological factors, both biological and molecular, affecting the pathogenicity of murine C-type viruses of the ecotropic and recombinant MCF virus classes are under study. In general, the MCF viruses which are thymus-derived are also thymotropic and lymphomagenic and have certain T1 oligonucleotide patterns in common; MCF virus obtained from other sources do not replicate in thymus, do not induce or accelerate lymphoma, and possess a different class of distinguishing oligonucleotide patterns. Biological characterization is in progress of viruses which are derived by transfection of DNA recombinants constructed from restriction enzyme fragments of cloned DNA of a lymphomagenic MCF virus and of AKR ecotropic virus. These viruses are being tested for host range, tissue culture plating efficiency, lymphomagenicity, in vivo replication, etc. in attempts to identify biological activities controlled by specific viral gene regions. Ecotropic viruses which are leukemogenic all replicate efficiently in vivo and regularly induce formation of MCF viruses; certain of these viruses are also being studied for the effects of interchanging portions of the viral genomes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00285-01 LVD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Studies of factors regulating xenotropic murine leukemia virus expression

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Janet W. Hartley, Ph.D.	LVD, NIAID
Other:	Wallace P. Rowe, M.D.	LVD, NIAID
	Christine A. Kozak, Ph.D.	LVD, NIAID

COOPERATING UNITS (if any)
H. C. Morse, R. A. Yetter, LMI, NIAID

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Viral Oncology Section

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.00	.17	.83

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Two strains of inbred mice, NZB and F/St, have been found to produce high levels of xenotropic murine C-type virus in lymphocytes, but they differ markedly in regulation and site of high virus expression. In NZB mice highest virus levels are found in bone marrow and spleen and expression is controlled by two independent dominant genes. In F/St mice, the highest frequency of xenotropic virus-producing cells is in thymus, a single locus for induction has been identified (on chromosome 1), and expression is recessive in most crosses with other inbred strains; suppression is controlled by a gene closely linked to H2K. The frequency of this type of control in other strains of mice and the mechanism of regulation are under study. These unique systems provide opportunities to study whether xenotropic virus sequences and their expression play a role in hematopoietic neoplasia.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00286-01 LVD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Tissue culture studies of genetic resistance to murine leukemia viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Janet W. Hartley, Ph.D.	LVD, NIAID
Others:	Wallace P. Rowe, M.D.	LVD, NIAID
	Christine A. Kozak, Ph.D.	LVD, NIAID

COOPERATING UNITS (if any)

Herbert C. Morse, III, M.D. (LMI, NIAID)

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National Institute of Allergy and Infectious Diseases, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	.33	1.42

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A host resistance gene has been identified in mice which specifically suppresses in vitro and in vivo replication of the MCF class of murine leukemia viruses. Designated R-Mcf, the gene is dominant for resistance, is located on chromosome 5 very close to the locus for hammer-toe (Hm), and is carried by DBA/1, DBA/2 and certain sublines of CBA (i.e., those related to CBA/H). In F1 hybrids between AKR females and males of various $Fv\text{-}1^n$ strains, the presence of the $R\text{-}Mcf^R$ allele results in significant delays in onset of spontaneous thymic lymphoma and in much reduced amplification of MuLV antigens and MCF virus in thymus. There is a similar but less dramatic effect on MCF virus accelerated disease in the F1's. The effect of this gene on other spontaneous and virus induced neoplasia is under study, along with other host genes which affect virus replication and spread.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00287-01 LVD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Chromosome localization of endogenous xenotropic MuLV proviral DNA sequences in inbred mice.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. David Hoggan, Sc.D.	Staff Scientist, LVD, NIAID
Other:	Christine A. Kozak, Ph.D.	LVD, NIAID

COOPERATING UNITS (if any)

M. A. Martin (LMM, NIAID)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
13/12	8/12	6/12

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Somatic cell hybrid cell clones prepared from Chineese hamster and mouse cells which contain different compliments of mouse chromosomes have been analysed for the presence of specific endogenous xenotropic env related DNA sequences. Continuing studies of the various somatic cell hybrid populations, by their originator and others using classical virological and genetic techniques in concert with Mendelian genetic studies of the various donor mouse strains have permitted the mapping of some of the many genes involved in the expression of endogenous xenotropic MuLV. One of these, the Bxv-1 locus common to BALB/c, AKR, C47BL, C57L, and C58 mice has been mapped to chromosome 1 in each of these strains. Our current studies have shown that at least 3 endogenous xenotropic related HindIII fragments are localized on chromosome 1 and analysis of these fragments are compatible with one of them being the Bxv-1 inducible locus, one being a MCF precursor sequence and the third being a sequence not known to give rise to known MuLV.
Further detailed studies of the DNA from the somatic cell hybrids in comparison to DNA from BALB/c mouse liver have allowed the assignments of at least 5 other endogenous xenotropic fragments to specific chromosomes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00288-01 LVD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Molecular cloning of endogenous xenotropic MuLV proviral DNA.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. David Hoggan, Sc.D.	Staff Scientist, LVD, NIAID
Other:	Charles E. Buckler	LVD, NIAID

COOPERATING UNITS (if any)

M. A. Martin and T. Theodore (LMM, NIAID)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5/12	4/12	1/12

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Ongoing studies of the organization and expression of endogenous xenotropic MuLV DNA sequences have highlighted the need for cloning each of the 28 defined HindIII fragments found in BALB/c mice. The availability of each of these fragments would allow us to better define the role of each proviral MuLV sequence along with its flanking DNA in virus expression. We have used the cosmid system to begin our studies and preliminary data suggests we have cloned the most prominent fragment (11.6 KB) which is not only found in BALB/c mice but is common to most mice tested.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00289-01 LVD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Organization of endogenous proviral DNAs of xenotropic murine leukemia viruses.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. David Hoggan, Sc.D.	Staff Scientist, LVD, NIAID
Other:	Charles E. Buckler	LVD, NIAID

COOPERATING UNITS (if any)

M. A. Martin (LMM, NIAID)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
11/12	6/12	5/12

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Restriction endonuclease analysis of genomic DNA from 24 inbred and 8 wild mouse strains have been carried out using the Southern blotting technique and a xenotropic reactive recombinant DNA probe. Except for 3 distantly related mus species the DNA from all mice tested contained numerous (18-28) xenotropic reactive HindIII fragments. Of these 4 were found common to all inbred and most wild mice tested. Because many common "bands" were found in closely related mice with fewer compating "bands" in more distantly related strains we were able to use these patterns to measure genetic relatedness between the various strains and to demonstrate an AKR genetic contamination of the high leukemic C3H/Fg strain. Such contamination is not found in the low leukemic C3H parent. Our studies of the internal organization of the various xenotropic related proviral sequences have allowed us to define at least 7 related but distinct families common to each mouse strain. To determine why some of these endogenous sequences are expressed as xenotropic virus, as MCF recombinant type virus or not at all in some mouse strains but not others, continues as the primary long range goal of this project.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00299-01 LVD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Genetic control and mapping of endogenous proviruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Christine Kozak, Ph.D.	(LVD, NIAID)
Other:	Herbert C. Morse, III, M.D.	(LVD, NIAID)

COOPERATING UNITS (if any)
Dr. Vincent Morris (Univ. of Western Ontario)

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National Institute of Allergy and Infectious Diseases, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.92	.41	.41

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Investigation of the genetic transmission of the ecotropic and xenotropic classes of murine leukemia viruses in inbred mice has led to the chromosomal mapping of 9 distinct loci. Studies with related laboratory mouse strains and Asian mice have been undertaken to describe the stability and wild mouse origin of specific loci and to identify additional genetic factors which alter in vivo virus expression. Most recently these studies have shown that the ecotropic V locus of CBA/J mice is allelic with that of several related strains. Other crosses are being used to describe the genetic basis for the great differences in induction efficiency shown by apparently allelic loci in DBA and SEA mice. The major xenotropic V locus of virus positive laboratory strains (Bxv-1) has now been identified in the Japanese mouse, M.m. molossinus. Finally, studies on the transmission of the endogenous B-type mouse mammary tumor virus in sexual crosses has shown that 3 unrelated mouse strains carry MMTV-related proviruses very near the MuLV locus Bxv-1.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00300-01 LVD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Genetic control of resistance to Friend virus in wild mouse populations.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Christine Kozak, Ph.D.	(LVD, NIAID)
Others:	Charles E. Buckler	(LVD, NIAID)
	M. David Hoggan, Sc.D.	(LVD, NIAID)

COOPERATING UNITS (if any)

M. Potter (NCI)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.83	.42	.41

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Japanese mouse, M.m. molossinus, carries a locus apparently identical to the Friend virus resistance gene, Fv-4, described originally in G strain mice. Sensitivity to Friend SFFV disease is now being examined in these mice to characterize Fv-4 and identify any other genetic factors which influence virus replication and the development of disease. To date, this analysis has resulted in the identification of a second locus on chromosome 5 which influences sensitivity to Friend virus, and the description of a sexual bias in susceptibility to disease. Other mouse species are currently being examined for sensitivity to virally-induced disease. More recent experiments have described the in vitro restriction of virus replication by cells carrying $Fv\text{-}4^r$. Genetic crosses are currently being tested to determine whether any of the novel MuLV reactive fragments identified by blot hybridization in $Fv\text{-}4^r$ mice are associated with resistance.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00301-01 LVD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Somatic cell genetic studies on endogenous proviruses.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Christine Kozak, Ph.D.	(LVD, NIAID)
Other:	M. David Hoggan, Sc.D.	(LVD, NIAID)

COOPERATING UNITS (if any)
S. Staal (Johns Hopkins); Robert Callahan (NCI); Stephen O'Brien (NCI).

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National Institute of Allergy and Infectious Diseases, Bethesda, Maryland

TOTAL MANYEARS: 1.2	PROFESSIONAL: .6	OTHER: .6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The use of interspecific hamster-mouse somatic cell hybrids has greatly expanded our studies on the genetic control of the transmission and expression of murine leukemia viruses. Hybrids are being used to identify and chromosomally map specific genes involved in viral leukemogenesis, and to simplify their further analysis by constructing cell lines carrying isolated genetic elements from this complex multigene system. Recent studies have demonstrated that the ecotropic and recombinant MCF viruses require different cell-surface receptors to infect mouse cells; genes for these receptors have been mapped to different chromosomes. Receptor loci for mouse leukemia viruses are also being identified and mapped in other species using cat-hamster and human-hamster hybrids. Hybrids which lack receptors are being used to describe the patterns of expression characteristic of specific endogenous V loci. Finally somatic cell hybrids are being analyzed with molecularly cloned viral probes to describe the chromosomal location and organization of these proviral sequences.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00302-01 LVD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Congenic mouse strains carrying genes of importance to murine leukemia virus infection.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Wallace P. Rowe, M.D.	LVD, NIAID
Other:	Janet W. Hartley, Ph.D.	LVD, NIAID

COOPERATING UNITS (if any)

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National Institute of Allergy and Infectious Diseases, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
23/12	8/12	15/12

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A number of genes are being bred onto standard inbred mouse backgrounds, chiefly the NFS Swiss. These genes include ecotropic virus-inducing loci, virus-resistance genes, and linkage marker genes for chromosomal regions of particular interest. The V-loci congenics have permitted the initial demonstration that the virus-inducing loci were in fact viral genomes, have allowed characterization of the genetic control of their induction, have allowed mapping of some, have allowed biochemical identification of the proviruses from multi-copy high-virus strains, have allowed identification of the crucial importance of virus titer in early life for rate of subsequent leukemia and the general independence of leukemia experience on the source and chromosomal location of the endogenous provirus, have shown the crucial importance of maternal antibody in determining the life long viral phenotype, and have demonstrated the occurrence of germline reinsertions of viral genomes. Together, the congenics permit an unprecedented degree of genetic analysis and strain development for analysis of viral leukemogenesis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00303-01 LVD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
In vivo studies of genetic resistance to murine leukemia viruses and virus-induced neoplasms.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Wallace P. Rowe, M.D.	LVD, NIAID
Other:	Janet W. Hartley, Ph.D.	LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Viral Diseases

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Viral Oncology Section

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
23/12	12/12	11/12

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The genetic basis of tumorigenic responses to endogenous or inoculated C-type viruses is being studied in a variety of strain crosses. The major determinants of spontaneous leukemias in hybrids with AKR have been shown to be the endogenous ecotropic virus inducing loci, the Fv-1 gene, and the Rmcf gene. In mice inoculated with MCF 247 virus, inheritance of an ecotropic virus-inducing locus of major importance for leukemogenicity. The Rmcf gene, which specifically inhibits infectivity of MCF viruses, is a strong determinant of leukemogenicity by MCF 247 and Friend helper virus and is possibly a minor determinant of ecotropic Moloney and Gross virus leukemogenicity. The effect of this gene on a variety of retroviral diseases is under study, including spontaneous B-cell lymphomas in high virus congenic mice, mice inoculated with erythroblastic or myeloid leukemia-inducing viruses, and mice inoculated with a neuroparalytic retrovirus.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00304-01 LVD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Hematologic and genetic studies of malignancies induced by Friend helper virus (F-MuLV).

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jonathan Silver, M.D.	LVD, NIAID
Others:	Torgny N. Fredrickson, D.V.M., Ph.D.	LVD, NIAID
	Herbert C. Morse, III, M.D.	LVD, NIAID
	Christine Kozak, Ph.D.	LVD, NIAID
	Charles Buckler	LVD, NIAID

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Friend MuLV is a broad-spectrum leukemogen which can induce erythroid, myeloid and lymphoid leukemias depending in part on the strain of mouse inoculated. Thus, Balb/c mice develop erythroleukemia (incidence>90%, latency~80 days), while C57BL/6 mice develop myeloid and lymphoid leukemias (incidence~90%, latency>200 days). This striking difference does not appear to be due to differences in F-MuLV replication. To identify mouse genes involved in determining the latency and type of leukemia induced by F-MuLV, we are studying the susceptibility of F1, first and second backcross mice, congenic strains, and recombinant-inbred strains of mice derived from Balb/c and C57BL/6. Preliminary results indicate that the C57BL/6 phenotype (late lymphoid and myeloid leukemias) is determined by 1 or 2 dominant genes which are distinct from previously identified Friend virus resistance genes including H-2, Fv-1 and Fv-2. Attempts to map these new genes are underway. We are also studying whether the leukemias induced by F-MuLV are clonal, and if so what cell lineages are involved in the leukemic clone, and when in the natural history of the disease clonality arises. Two techniques are being used: (1) "Southern blotting utilizing an ecotropic-specific viral DNA probe, and (2) determination of pgk isoenzyme type of tumor vs. normal tissue in female mice heterozygous for the X chromosome-coded pgk enzyme.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00305-01 LVD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Studies on the mechanism of Fv-1^{b} inhibition of AKR thymomagenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jonathan Silver, M.D.	LVD, NIAID
Other:	Herbert C. Morse, III, M.D.	LVD, NIAID

COOPERATING UNITS (if any)

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National Institute of Allergy and Infectious Diseases, Bethesda, Maryland

TOTAL MANYEARS: 4/12	PROFESSIONAL:	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Genetic studies with AKR mice have shown that the Fv-1^{b} gene strongly inhibits the development of thymomas. This gene restricts the expression and spread of N-tropic viruses including the endogenous AKR ecotropic virus and N-tropic MCF viruses. Congenic AKR.Fv-1^{b} mice express about 10- to 100-fold less ecotropic virus in the thymus than AKR mice, and do not develop thymomas at 6 to 12 months of age like their AKR counterparts. To study whether the Fv-1^{b} effect in vivo is mediated by thymocytes or thymic stroma, reciprocal subcutaneous thymus graft and bone marrow chimeras are being constructed between AKR/Cum and AKR.Fv-1^{b} mice. The former strain is Fv-1^{n} and carries the Thy 1.2 marker on thymocytes, allowing one to distinguish thymocytes derived from AKR/Cum bone marrow in AKR.Fv-1^{b} recipients and vice versa. Preliminary results indicate that thymic stroma does not mediate the restrictive Fv-1^{b} effect in mice carrying AKR and AKR.Fv-1^{b} subcutaneous thymic grafts. Further experiments involving bone marrow chimeras are in progress.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00306-01 LVD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies on the biology of mousepox and development of a specific serologic test for ectromelia virus.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Gordon D. Wallace	Senior Investigator, LVD, NIAID
Other:	Mark Buller	Visiting Associate
	Bernard Moss	Chief, Molecular Struc. Sec., LBV, NIAID
	Herbert Morse	Chief, Vir. and Cell. Immunology, LMI, NIAID

COOPERATING UNITS (if any)
The Australian National University

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Viral Oncology Section

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, Bethesda, Maryland

TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.6	OTHER: 0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Because of recent epizootics of mousepox at NIH and other biomedical research institutions in the U.S., research was undertaken to develop a simple, sensitive and specific test to detect antibody to ectromelia virus, and to develop an in vitro assay for infectious virus. In addition, a study of biological and biochemical markers of virus strains in various strains of inbred mice as correlates of epizootiology and pathogenesis of mousepox are in progress. This basic biological information and diagnostic methodology are considered essential for a rational approach to the control and prevention of mousepox, which threatens serious disruption of biomedical research in the U.S.

PHS-6040
(Rev. 10-76)

LABORATORY OF MICROBIAL STRUCTURE AND FUNCTION
Rocky Mountain Laboratories
Hamilton, Montana
1982 Annual Report
Table of Contents

Z01 AI Project Number		Page
Summary		27-1
00065-09	Antigens and Classification of Rickettsiae -- Anacker	27-9
00071-11	Purification and Activities of Biologically Active Substances from Bordetella pertussis -- Munoz (Project suspended while on sabbatical)	27-10
00076-24	Mechanisms of Immunopotentiation by Components of Microbes -- McLaughlin (terminated)	27-11
00182-04	Biochemical and Genetical Mechanisms of Obligate Intracellular Parasitism -- Williams	27-12
00183-04	Structural and Functional Relationships of Bacterial Antigens in the Immune Response -- Williams	27-13
00193-03	Gonococcal Surface Components: Structure and Function -- Swanson	27-14
00194-03	Molecular Genetics of Neisseria gonorrhoeae -- Mayer	27-15
00195-03	Molecular Aspects of Mycoplasma-Eukaryote Interactions -- Liss	27-16
00196-03	Bordetella pertussis: Outer Membrane Structure and Function -- Peppler	27-17
00215-02	Mechanisms of Action of Beta-lactam Antibiotics -- Barbour (terminated)	27-18
00216-02	Immunochemistry of Chlamydial Surface Antigens -- Caldwell	27-19
00230-01	Virulence-Associated Factors of Rickettsia rickettsii -- Anacker (new project)	27-20
00231-01	Biology of Relapsing Fever Borrelia spp. -- Barbour (new project)	27-21
00232-01	Biology of Ixodes spp. Tick Spirochetes -- Barbour (new project)	27-22

Table of Contents (Continued)

Z01 AI Project Number		Page
00233-01	Structural Analysis of the Major Outer Membrane Protein of *Chlamydia trachomatis* -- Caldwell (new project)	27-23
00234-01	Macrophage Interaction with Intracellular Parasites -- Hackstadt (new project)	27-24
00235-01	Structure and Function of Gonococcal Lipopolysaccharide -- Hitchcock (new project)	27-25
00236-01	Studies on the Biology of Contagious Equine Metritis Organism -- Hitchcock (new project)	27-26
00237-01	Structural Analysis of Gonococcal Outer Membrane Proteins -- Judd (new project)	27-27
00238-01	*Bordetella pertussis* and *Bordetella bronchiseptica*: Outer Membrane Structure -- Peppler (new project)	27-28
00239-01	Gonococcal Surface Proteins' Immunochemical Characteristics -- Swanson (new project)	27-29

Annual Report
Laboratory of Microbial Structure and Function
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1981, to September 30, 1982

RESEARCH HIGHLIGHTS

During the past year this laboratory has recorded several observations that provide important clues to the strategies employed by pathogenic bacteria to cope with their hosts. Major emphasis has been on bacterial surface components regarding their structures and functions. This effort seems to be slowly yielding information about the "personality-shaping" (i.e., surface-exposed) elements of bacteria, but results suggest that high order variability is the rule for many organisms' surface components, particularly extracellular parasites. Attainment of intracellular location and mechanisms that thwart usual intracellular killing devices are integral parts of the pathogenicity/virulence of obligate intracellular parasites. The bacterial components participating in these events are being studied in chlamydia, spotted fever rickettsiae, and *Coxiella burnetii*. Some of the studies which have been carried out during the past year by the LMSF staff to elucidate structure and functional relationships of bacterial pathogens are described in the following.

Chlamydial outer membrane proteins: structure and immunochemistry. Harlan Caldwell continued his characterization of the chlamydial major outer membrane protein's (MOMP's) structure and function. Among all *Chlamydia trachomatis* serotypes, antigenic relatedness of their MOMPs can be demonstrated by immunoblotting with antisera raised against isolated MOMPs. The same antisera will protect HeLa cells from infection with chlamydia of the homologous serotype. These immunoblotting results contrast with immunofluorescence or infectivity-reduction results with the same anti-MOMP sera where serotype-specific reactions were observed; they also contrast with immunoblotting results with sera raised against whole, fixed chlamydial elementary bodies (EBs). These antisera contain a mixture of species-specific and serotype-specific antibodies which can be differentiated by dilution of the serum, etc. Both the cross-reactive and the unique antigens are being located in particular MOMP fragments generated by protease cleavage. The differences and similarities in MOMP antigens correlated well with the peptide mapping results obtained by Ralph Judd and Harlan Caldwell on chlamydia MOMPs. A number of ^{125}I-peptides generated by chymotrypsin seem to represent elements that have been "conserved" im MOMPs of several chlamydia species; however, analysis of surface-^{125}I-labeled peptides revealed uniqueness for each of the serotypes but with some sharing between particular serotypes (L2 and D, H and I, etc.). This study, which has employed both analytical thin-layer and (potentially preparative) HPLC peptide characterization, is aimed at isolation of peptides responsible for the various surface-exposed antigens of chlamydiae. The neutralization of chlamydial infection with anti-MOMP antiserum observed by Caldwell is of interest from several vantages. First, since affinity-purified anti-MOMP IgG was neutralizing, a function for the MOMP in the pathogenesis of chlamydial infection can be inferred. Second, the antiserum-neutralizing step in chlamydial infection appears to be one subsequent to endocytosis (phagocytosis) of the chlamydial EB. Prevention of fusion between phagosomes with lysosomes has previously been proposed as a mechanism whereby chlamydia could escape destruction by lysosomal enzymes. That anti-MOMP serum prevents chlamydial infection subsequent to internalization of the antibody-coated organisms in vesicles suggests that MOMP might function in the inhibition of phagosome-lysosome fusion. Third, Fab fragments (from anti-MOMP IgG) do not neutralize infectivity of chlamydia, but they should be useful reagents, along with monoclonals being developed, for probing both extracellular and intracellular aspects of chlamydial multiplication, degradation, etc.

Borrelial outer membrane proteins: *structure and expression*. *Alan Barbour* studied the outer membrane proteins of *Borrelia hermsii* with special interest being directed toward those proteins whose antigenicities are responsible for the serotypes previously discovered and described by *Herb Stoenner*. Three of the serotypes (7, 14, and 21) which occur most frequently in infected mice were used as prototypes for development of both polyclonal and monoclonal reagents directed against the serotype antigens. These were found to be an MOMP of *B. hermsii*, as defined by its predominance, its surface-iodinatability, etc. The apparent subunit MWs of this outer membrane protein (designated protein I or pI) varies among different serotypes. Some antigenic moieties are shared among different pIs; but surface reactivities of different pIs are quite restricted as shown with both polyclonal and monoclonal antibodies. During in vitro growth, *B. hermsii* may cease expression of typical pI constituents and, instead, there appears a "core" protein (previously defined by *Stoenner*) whose subunit size is only a fraction of that for the pI of the organisms used as inocula. The Borrelia pIs of "core," 7, 14, and 21 serotypes were analyzed by ^{125}I-peptide mapping by *Judd* and *Barbour*; these were nearly identical in their chymotryptic peptide maps, but each bore unique peptides. Of particular interest is the relationship between "core" and other (7, 14, etc.) serotype pIs. The genetic mechanisms responsible for this change in outer membrane expression is being explored by *Barbour*, *Leonard Mayer* and *Claude Garon* who are presently characterizing the *B. hermsii* genome preparatory to cloning the DNA which codes for the outer membrane protein. Borrelia appears to be somewhat unusual in the high relative proportion of its DNA which is in covalently closed circular (plasmid) form. This organism has five size classes of plasmids; one or more appears to be lost during cultivation in vitro. A number of Borrelia's biological characteristics should make this organism a highly useful model for investigating expression of varying surface antigens by a pathogen, and several investigators from other institutions have consulted *Barbour* for help in their commencing studies on this organism (Foulds, NIAMMD, LBM; Simon, Cal Tech).

Lyme disease spirochete. *Alan Barbour* was also a key member of the group that discovered a spirochete in *Ixodes* ticks that is almost certainly the infectious agent causing erythema chronicum migrans (ECM) and the arthritic and/or neurologic syndrome called Lyme disease. The causative organism was discovered in ticks by *Willy Burgdorfer* (EB), was successfully grown in vitro by *Barbour*, and has been utilized in immunoblotting with sera from patients with ECM, etc., or from laboratory animals inoculated with the spirochete. Several findings from these studies are of interest including the apparent antigenic relatedness between this spirochete and *Treponema pallidum*, generation of anti-outer membrane protein antibodies in infected patients, production of monoclonal antibodies against various outer membrane of the ECM spirochete, etc.

Peptide analyses of outer membrane proteins. A key tool in evaluating structural diversity of outer membrane proteins has been ^{125}I-peptide mapping which has been done primarily by *Ralph Judd*. He has studied the following bacterial outer membrane proteins: *N. gonorrhoeae* proteins I (about 15 strains), proteins II (three strains with several PIIs in each strain), protein III; *B. hermsii* pI, pII, pIII and p_{core} for several intrastrain variants; *C. trachomatis* MOMPs from ten strains, and V8-protease fragments from same MOMPs; and *Bordetella pertussis* MOMPs. In addition, ^{125}I-peptide mapping is now being used for several viral proteins (Aleutian Disease Virus, murine leukemia viruses). This 2-dimensional method for displaying and analyzing radioiodinated peptides is a powerful tool for "finger-printing" proteins which are difficult to prepare free of contaminating membrane components and allow for relatively rapid comparisons of numerous proteins. This method has been coupled with high-pressure liquid chromatographic (HPLC) separation of both ^{125}I- and ^{14}C-labeled outer membrane proteins and, surprisingly, the relatively simple 2-D method of displaying radiolabeled peptides seems more sensitive and just as reproducible as HPLC techniques. HPLC is superior, however, in recovery of desired peptides, and it is this attribute which makes HPLC attractive for studies intended for the near future with both chlamydial and gonococcal outer membrane peptides.

Gonococcal outer membrane proteins: *structure and immunochemistry*. Several gonococcal surface constituents were studied by *John Swanson*. Gonococci of any given strain were found to express none, one, or more (up to five) protein II (PII) constituents, each of which has a distinctive influence on colony opacity, SDS-PAGE migration characteristics, subunit size, and ^{125}I-peptide map pattern; all PIIs exhibit protease sensitivity, surface exposure, and several other characteristics including a number of "identical" ^{125}I-peptides. Each protein II seems to be relatively unique in the surface-exposed antigens it displays on gonococci; however, many antigens that seem "buried" in gonococcal outer membranes seem to be cross-reactive and probably represent "conserved" portions of the basic protein II molecule. These conclusions are based on immunoprecipitation and immunoblotting experiments with sera raised against whole PII^{+} gonococci or with sera raised against isolated PII bands (from SDS-PAGE). Outer membrane protein III constituents differ from PIIs in many ways including: protease insensitivity, totally conserved ^{125}I-peptides (*Judd*) and antigens among all strains of gonococci assayed, identical subunit MW among diverse strains. It was also found that PIII occurs in a heteropolymeric complex with protein I (the major serotype antigen and a porin). Protein III is surface exposed on all gonococci; the significance of such a "common" antigen on gonococci is not clear. Variations have been demonstrated for pili among different strains and among variants within a single strain; these variations are in apparent subunit MW and up to six different subunit MW forms have been found in a single strain. These observations derive from studies on single gonococcal colonies after SDS-PAGE and immunoblotting with antiserum raised against isolated pili or a particular cyanogen bromide fragment of pili. Use of this immunoblotting procedure allowed visualization of subunits of pili present on about 5 x 10^{3} organisms; this high sensitivity allows study of pili in relatively minute subpopulations of gonococci and should be very useful in terms of detecting minor changes in pilus form which may occur during gonococcal growth in vitro or in vivo.

A beginning attempt was also made to identify, characterize, and purify those surface-exposed, antigenic portions of protein II molecules. This is toward elucidation of biological properties of gonococci that are endowed by different protein II constituents and is designed to provide immunological reagents capable of probing those properties.

Lipopolysaccharide structure variants. *Penny Hitchcock* began studies on heterogeneity or diversity of LPS structure among different gonococci. For these studies, she devised methods that are developing as informative tools for elucidating LPS forms in whole bacterial lysates. The method includes use of proteinase K to completely hydrolyze bacterial proteins (at 60°C in SDS), resolution of proteinase K-resistant material in SDS-PAGE and display of LPS constituents by means of selective silver staining. Striking examples of differing LPS structures have been found among different gonococci, among different Serratia preparations (with David Morrison), among *B. pertussis* and *bronchiseptica* strains (*Peppler*), in Phase I versus Phase II *C. burnetii* (*Williams*), and among the classic series of Salmonella LPS mutants. Separation of LPS components and their subsequent demonstration is so discriminating that LPSs differing by a single heptose residue can be reliably differentiated. Use of similar techniques has yielded results that raise several questions about the technique of SDS-PAGE as a tool for separating various components. Evidence of protein-protein and protein-LPS interactions that are not disrupted by SDS and interference in staining one "class" (e.g., LPS) of moieties by the presence of another kind of constituent (e.g., protein) at the same location in a gel, have been obtained; and these findings raise important questions concerning criteria by which bacterial constituents are adjudged "pure," etc.

Contagious equine metritis organism. Studies on the gram-negative bacterium which causes contagious venereal infections in horses were started by *Penny Hitchcock*. Initial work included devising improved solid and liquid media that will satisfactorily propagate the organisms from several strains (both streptomycin sensitive and resistant). Characterization of the outer membrane proteins, LPS, and colony opacity phenotypes are underway for this organism that has received little experimental attention in the past. This organism will be studied to define its virulence/pathogenicity related components and its interactions with equine cells and tissues.

Metabolism of Coxiella burnetii Metabolism of *C. burnetii* was studied by *Ted Hackstadt* and *Jim Williams* both to understand the acidity-stimulated transport systems of this organism and to provide information for design of an axenic medium for propagating this obligate intracellular parasite. In the latter regard, recent studies have shown that macromolecular synthesis of small numbers of proteins by *C. burnetii* has been achieved in cell-free medium. The acidic pH dependence of most transport and metabolic systems in *C. burnetii* were exploited in probing mechanisms whereby these organisms maintain their millieu interior. This "acidophilic" intracellular pathogen maintains a pH gradient across its covering membranes with its internal pH slightly higher than the surrounding environment. Acceptable intracellular pH is maintained in the absence of metabolic energy, and this may partially explain the organisms' stability in nature.

Macrophage-bacterial interactions. *Ted Hackstadt* also started studies aimed at understanding relationships between macrophages and bacterial agents which parasitize these usually phagocytic, protective scavenger cells. Studies are underway on macrophages in combination with *C. burnetii*, with *Chlamydia psittaci*, or with mycoplasmas. Each of these organisms has a different lifestyle regarding the macrophage host; mycoplasmas remain outside the cell, psittaci chlamydia inhibit phagosome-lysosome fusion, and *C. burnetii* multiply in fused phago-lysosomes. Neither *C. burnetii* nor *C. psittaci* induce an oxidative burst in macrophages on phagocytosis, but the macrophages can mount such a reaction to subsequent phagocytic events (of staphylococci). Use of mycoplasmas resulted in demonstration that these organisms can inhibit the oxidative burst of mouse or guinea pig macrophages which are inhibited in their subsequent reactions to a variety of chemical and microbial agents.

Molecular genetic studies. Restriction endonuclease mapping of two gonococcal R plasmids has been done by Leonard Mayer. The plasmids contain a transposon (Tn3), but regions from both ends of the Tn3 are missing. In addition, the plasmid seems to be unusually deficient in restriction sites as compared to the Tn3-derived portion. These studies utilized a newly-developed method for labeling only one end of a DNA molecule after its cleavage by the enzyme Ava I; this method should be of use in DNA sequencing. Molecular cloning of chlamydia and borrelia genomes were carried out to construct a gene bank for each organism. These are being screened for production of outer membrane proteins of these pathogens. Attempts are underway to characterize several facets of borrelia DNA including genome size and the coding capabilities of the five distinct size classes of plasmid that have been demonstrated in these organisms.

Mycoplasmas and their viruses. Variation of surface components was demonstrated by Alan Liss with regard to Mycoplasma pulmonis (a rodent pathogen) and a M. pulmonis-infecting phage, MVMP-1, which was discovered by Liss and co-workers. A change in colony opacity phenotype of the mycoplasma was accompanied by a change in their susceptibility to infection with MVMP-1; this change is also accompanied in changes in the restriction endonuclease maps generated from whole mycoplasma DNA.

Bordetella pertussis and bronchiseptica. Mark Peppler continued a systematic appraisal of several characteristics of B. pertussis and B. bronchiseptica to both document correlations between composition and phenotype and to construct isogenic sets that will be useful for dissecting the role of various of the bacterium's components in causing infections in animal models. This is a difficult task; abundant descriptive work on B. pertussis has been done, but much of it is not very useful for definition of pathogenesis and/or virulence-related characteristics of these organisms. The bacterium is both quite versatile as a producer of biologically active substances and can vary its surface protein constituents in ways that are currently too complex for analysis. Therefore, isogenic sets of organisms differing by single, defined ways are essential for documenting structural and functional relationships with this bacterium. To date, a number of changes with apparent rates comparable to classical mutation rates (10^{-7} or less) have been defined, namely changes from domed, hemolytic (D^+H^+) to flat, nonhemolytic (D^-H^-). This is a fascinating conversion or change which involves a number of components/products that exhibit coordinate "on-off" expression. During the past year, Peppler has developed several reagents and adapted methods for evaluating changes in LPS structures from B. pertussis. These include monoclonal antibodies directed against LPS, a repertoire of pyocins which use different LPSs as receptors, and SDS-PAGE silver stain techniques. In addition, he has defined a new phenotype (ability to grow on nutrient agar) whose relation to LPS form is being studied.

Spotted fever rickettsial antigens. Antigenic constituents of Rickettsia rickettsii have been studied by Bob Anacker to discover which one(s) are common to diverse strains and, hence, constitute a "group" antigen for the spotted fever group rickettsiae. A group antigen was found and appears very similar or identical to previously-described constituents (erythrocyte sensitizing substance and complement fixing antigen). A number of *R. rickettsii* antigens reacted with sera from patients who had Rocky Mountain spotted fever; some sera protected mice from lethal challenge with large numbers of rickettsiae, and these seemed to recognize two particular antigens by crossed-immunoelectrophoretic assay. Beginning experiments designed to define "virulence-factors" of *R. rickettsii* are underway, and these will focus on surface components of rickettsiae that exhibit different (P less than 0.01) virulence by temperature rise and scotal swelling assays in guinea pigs.

Technical developments. Three new techniques evolved from older methods and should be of interest to a number of other workers. The first was developed by *Leonard Mayer* and involves cleavage of DNA with an endonuclease (such as Ava I) that has an asymmetric cleavage site. One end of the DNA molecule can be selectively labeled with a desired nucleotide triphosphate (radiolabeled) after using "fill in" synthesis by DNA polymerase or reverse transcriptase. The second is a modification of the silver staining techniques for visualizing proteins and polysaccharides in polyacrylamide gels. This modification, developed by *Penny Hitchcock*, allows for preferential staining of bacterial lipopolysaccharides (proteins don't stain) or protein + polysaccharide staining, as desired. A single gel can be sequentially stained, decolorized, restained with each staining cycle designed to display different SDS-PAGE separated constituents. This method, coupled with proteinase K digestion of SDS-lysates of whole bacteria, allows rapid evaluation and comparison of bacterial lipopolysaccharides. The third method developed is a slight modification of existing immunoprecipitation methodology and involves exposure of intact bacteria to antiserum and subsequent immunoprecipitation of the surface-exposed antigens (bacterial) + IgG complexes. This was developed by *John Swanson* and permits ready evaluation of gonococcal or other gram-negative bacterial outer membrane antigens with sufficient surface exposure to allow their combination with added antibody molecules.

Collaborative efforts. Several collaborations between LMSF staff and those from other labs at RML were particularly productive the past year. *Alan Barbour's* collaboration with *Willy Burgdorfer* (EB) led to the discovery of the agent most certainly causative of ECM and Lyme disease. Willy discovered the spirochete in the Ixodes tick gut and Alan's immunochemical analyses showed the presence of antibodies in sera from patients recovered from ECM and Lyme disease, etc. Another very fruitful collaboration yielded the successful cloning of the major portion of the Aleutian Disease Virus' genome by *Leonard Mayer* and *Marshall Bloom* (LPVD). That result should be quite useful for deciphering genetic aspects of this virus. Several collaborative efforts developed with members of the electron microscope laboratory, and these were quite fruitful.

Annual Report
Laboratory of Microbial Structure and Function
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1981, to September 30, 1982

ADMINISTRATIVE REPORT

Staff changes during the reporting year include the following: Additions - Claes-Otto Kindmark (Visiting Scientist - December 1981 to September 1982); departures - Charles McLaughlin (Expert), Alan Liss (Expert), Ralph Judd (Staff Fellow), Kenichi Amano and Mitsuru Watanabe (Visiting Fellows). There were three departures from the technical staff (Vern Fadness, Bill Bickel, and Linda Perry) and no additions. Summer students included Daryl Warder (Johns Hopkins medical student), Ramona Heiland (enrolled in University of Utah Medical School), and Todd Damrow (University of Montana). John Munoz is on foreign assignment (one year) to the Walter and Eliza Hall Institute for Medical Research, Melbourne, Australia.

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Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1981, to September 30, 1982

HONORS AND AWARDS

Journal Editorial Boards:

J. Swanson - Journal of Bacteriology and Infection and Immunity

Manuscripts from Infection and Immunity, Journal of General Microbiology (London), Journal of Immunology, Journal of Infectious Diseases, Science and Sexually Transmitted Diseases were also reviewed by members of LMSF staff.

Professional Posts:

A. Liss was a Faculty Affiliate, Department of Microbiology, University of Montana. He served as Chairman of the Subcommittee for the Taxonomy of Pseudomonas Bacteriophage, International Committee for the Taxonomy of Viruses.

L. Mayer co-chaired a session at Mid-Atlantic Extrachromosomal Elements meeting, Ocean City, MD

Invited Lectures:

J. Swanson - Cell Surface Involvement Symposium, Ottawa, Canada
ASM Symposium on Bacterial Adherence, Atlanta, GA
University of Montana seminar, Missoula, MT
University of North Carolina seminar, Chapel Hill, NC
Pathogenic Neisseriae Conference, Montreal, Canada
International Congress of Microbiology Symposium on Outer Membranes of Gram-negative Bacteria, Boston, MA

A. Barbour - American Leptospirosis Conference, Amherst, MA
Yale University School of Medicine, New Haven, CT

H. Caldwell - Fifth International Symposium on Human Chlamydial Infections, Lund, Sweden

Other Honors:

A. Barbour - Elected to membership in Infectious Disease Society of America.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00065-09 LMSF

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Antigens and Classification of Rickettsiae

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. L. Anacker	Res. Microbiologist	LMSF NIAID
OTHER:	R. N. Philip	(Retired) Medical Director	EB NIAID
	L. A. Thomas	Research Microbiologist	EB NIAID

COOPERATING UNITS (if any)
Dr. K. E. Hechemy, New York State Dept of Health, Albany, NY; Dr. C. M. Wilfert, Duke Univ Medical Center, Durham NC; Drs. K. T. Kleeman and J. N. MacCormack, North Carolina State Dept of Health, Raleigh, NC

LAB/BRANCH
Laboratory of Microbial Structure and Function

SECTION
Rickettsial Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	1.0	1.2

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are the determination of the nature and biological properties of rickettsial antigens and constituents and the development of procedures for classification of spotted fever group rickettsiae. The serological relationship of the spotted fever group antigen and the erythrocyte sensitizing substance was determined by tandem crossed immunoelectrophoresis. Evidence of the structure and chemical nature of the group antigen was obtained by a combination of immunological and chemical methods. Antibodies to particular Rickettsia rickettsii antigens were demonstrated in the sera of spotted fever patients by crossed immunoelectrophoresis.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00071-11 LMSF

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Purification and Activities of Biologically Active Substances from Bordetella pertussis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. J. Munoz	Res. Microbiologist	LMSF NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

Pertussis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Project suspended while on sabbatical.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00076-24 LMSF

PERIOD COVERED
October 1, 1981, to May 10, 1982

TITLE OF PROJECT (80 characters or less)
Mechanisms of Immunopotentiation by Components of Microbes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. A. McLaughlin	Expert	LMSF NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.5	OTHER: 0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this project to delineate, at the cellular and molecular levels, the mechanism of action of microbial components in immunoregulation. In the past year the main research activities have been investigations of the interaction of endotoxin with lipoproteins, phospholipids, and monoclonal antibodies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00182-04 LMSF

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Biochemical and Genetical Mechanisms of Obligate Intracellular Parasitism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. C. Williams	Scientist	LMSF NIAID
OTHER:	T. Hackstadt	Staff Fellow	LMSF NIAID

COOPERATING UNITS (if any)
Dr. Thomas McCaul, Dept Med Microbiol, London School of Hygiene and Trop Med, Keppel Street, London WCIE 7HT, England

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION
Rickettsial Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.0	0.8

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective is to characterize the biochemical and genetic mechanisms of obligate intracellular parasitism between the eukaryotic host and prokaryotic parasite. Our recent studies have suggested that a key feature of intraphagolysosomal growth of Coxiella burnetii, the etiological agent of Q fever, is a membrane structurally and functionally compatible with the acidic and otherwise hostile environment of the phagolysosome. Thus, we have proposed a pathogenic mechanism for C. burnetii based on regulation of microbial metabolism by H^+-dependent stimulation of cell function. We have continued to probe the molecular mechanisms of this pH dependency and have demonstrated that C. burnetii behaves similarly to other acidophilic bacteria in that it maintains an intracellular pH more alkaline than the environment and that this pH gradient (ΔpH) is used to energize glutamate transport. The maintenance of the ΔpH is at least partially energy dependent. The stability of C. burnetii in the environment may be explained, in part, by the lack of a requirement for metabolic energy to maintain acceptable intracellular pH values in neutral or alkaline environments.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00183-04 LMSF

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Structural and Functional Relationships of Bacterial Antigens in the Immune Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. C. Williams	Scientist	LMSF NIAID
OTHER:	M. G. Peacock	Microbiologist	EB NIAID
	T. A. Damrow	Graduate Student	LMSF NIAID
	K. Amano	NIH Visiting Fellow	LMSF NIAID
	L. A. Thomas	Research Biochemist	EB NIAID
	C. O. Kindmark	NIH Visiting Scientist	LMSF NIAID

COOPERATING UNITS (if any)

Dr. T. McCaul, London School of Hygiene and Trop Med, England; Dr. F. T. Koster, Div Infectious Dis, Univ of New Mexico School Med, Albuquerque

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION
Rickettsial Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 6.3	PROFESSIONAL: 3.8	OTHER: 2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective is to characterize the protective antigens and toxic products of Coxiella burnetii. Structure and functional aspects of the immunomodulation by surface components of C. burnetii are being analyzed in vivo and in vitro as candidates for subunit vaccines. We have shown that C. burnetii adsorbs normal serum acute phase proteins to its surface which may enhance opsonization by phagocytic cells; surface polysaccharide is implicated as a virulence factor of phase I cells whereas phase II, avirulent cells have lost this surface component; mouse monoclonal antibodies directed to surface polysaccharide (IgG2a) shows specificity for phase I cells whereas monoclonal antibodies (IgG2b) directed to surface protein shows specificity for phase II cells; an enzyme linked immunoabsorbent assay has been developed for the detection of early antibody (phase II immunogen) and late antibody (phase I immunogen); subfractionation of C. burnetii cells into peptidogylcan (PG) insoluble fractions have shown that the phase II immunogen is a protein covalently bound to the PG whereas the phase I immunogen is a soluble polysaccharide; and phase conversion (I to II) in C. burnetii is similar to the smooth to rough variation described for gram-negative bacteria.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00193-03 LMSF

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Gonococcal Surface Components: Structure and Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Swanson	Chief	LMSF NIAID
OTHER:	O. Barrera	Microbiologist	LMSF NIAID

COOPERATING UNITS (if any)
Dr. G. Schoolnik, Stanford University, Stanford, California

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A major focus for this project during the past year has been the protein II family of constituents of gonococcal outer membranes. One or more of these proteins may be present: all are heat modifiable, susceptible to exogenous proteases, and have similar ^{125}I-peptide map patterns. However, at least five different protein(s) II may be found in a single strain of gonococci, and each of the protein II constituents exhibits slightly different properties such as subunit size, dependence on 2-mercaptoethanol and heat for electrophoretic migration characteristics, and correlation with colonial opacity. Variation in pilus subunit molecular weights has also been studied using pilus-specific antisera. Many different subunit molecular weight forms of pili can be found within given strains. These diverse strains also differ from one another in the apparent sizes of their pilus subunit variants. Changes in pilus subunit form do not accompany changes in protein II constitution. Although not absolutely correlated, changes in piliation phenotype (P$^+$ and P^{++}) are usually accompanied by changes in pilus subunit molecular weight.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00194-03 LMSF

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Molecular Genetics of Neisseria gonorrhoeae

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. W. Mayer	Expert (Microbiology)	LMSF NIAID

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.9	0.9	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The emphasis of this project is to use molecular genetics, including recombinant DNA techniques, to understand the pathogenesis of gonorrhea and some other infectious diseases. We have characterized one of the gonococcal R-plasmids by determining a physical map of many restriction endonuclease sites. While characterizing this plasmid, we also developed a method to specifically end-label DNA fragments by using certain enzymes which make asymmetric cleavages of the DNA. We have made gene banks of gonococci and chlamydia which are being screened for the production of surface proteins. We have also characterized the genome of Borrelia hermsii with regard to G+C content, genome size, and plasmid DNA content. One or more of the 5 plasmids in B. hermsii appears to be lost during cultivation in vitro.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00195-03 LMSF

PERIOD COVERED
October 1, 1981, to July 31, 1982

TITLE OF PROJECT (80 characters or less)
Molecular Aspects of Mycoplasma-Eukaryote Interactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Liss	Expert (Molecular Biology and Bacteriology)	LMSF NIAID

COOPERATING UNITS (if any)
Dr. G. Cassell, University of Alabama, Birmingham,
S. F. Hayes, Electron Microscopy Section, Rocky Mountain Laboratories

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project's long-term goal is to determine the relationship between mycoplasmas and their eukaryotic hosts. Current emphasis is on elucidating the mycoplasmal surface architecture. Acholeplasma laidlawii whole cell polypeptide profiles, using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), have been determined. The profiles are not changed when A. laidlawii is infected with either MVL51 (a filamentous virus) or MVL2 (an enveloped virus). Mycoplasma pulmonis, etiological agent of murine respiratory mycoplasmosis (MRM), is also being studied. A virus, MVMP-1 has just been isolated. It has icosahedral head morphology and a possible tail structure. Characterization of MVMP1 and its M. pulmonis host are in progress.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00196-03 LMSF

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Bordetella pertussis: Outer Membrane Structure and Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Peppler	Expert (Microbiology)	LMSF NIAID

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 1.4	PROFESSIONAL: 0.7	OTHER: 0.7

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project's purpose is to define the biochemical composition of the major surface constituents of Bordetella pertussis and their relationship to the pathogenicity and epidemiology of whooping cough. Techniques have been developed which allow for the controlled selection, from a single strain, of the three major, stable phenotypes of B. pertussis. Isogenic sets of cloned phenotype variants are compared for differences in SDS-PAGE profiles after Coomassie brilliant blue staining for protein, extrinsic labeling by ^{125}Iodine and/or silver staining for lipopolysaccharide. The three phenotypes possess characteristic SDS-PAGE profiles, colonial morphologies on BGA, growth characteristics on nutrient agar and antibiotic and oleic acid sensitivity profiles. Lipopolysaccharide variants have been characterized and their role in phenotype dynamics is being studied. In addition, biochemical characterization of FHA and pertussigen has begun. Analysis of specific mutants and their products can better define the genetic basis of virulence in B. pertussis for furthering understanding of pathological and immunological mechanisms of whooping cough.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00215-02 LMSF

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Mechanisms of Action of Beta-lactam Antibiotics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. G. Barbour	Senior Staff Fellow	NIAID LMSF

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

During the past year, work under this project number was terminated. Studies, which were outlined in the 1981 Annual Report on the penicillin-binding proteins of Borrelia hermsii and of Chlamydia trachomatis, were completed.

PHS-6040

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00216-02 LMSF

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Immunochemistry of Chlamydial Surface Antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. D. Caldwell	Expert (Microbiology)	LMSF NIAID

COOPERATING UNITS (if any)
Dr. J. Schachter, University of California Medical Center, San Francisco

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	0.7	1.1

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major outer membrane protein (MOMP) of several Chlamydia trachomatis serotypes was isolated by preparatory SDS-PAGE and used to raise hyperimmune rabbit antisera. The specificity of these antisera is being analyzed by: (i) rocket immunoelectrophoresis and immuno-blotting of SDS-denatured MOMP, (ii) indirect immunofluorescence of purified elementary bodies (EBs), and (iii) in vitro neutralization of chlamydial infectivity. By immunoblotting analysis with anti-MOMP sera, all C. trachomatis MOMPs showed strong immunological cross reactivity demonstrating that SDS-insensitive species-specific antigens are the primary determiners measured by this technique. In contrast, these same antisera showed considerable serotype specificity when tested by both immunofluorescence staining of EBs and neutralization of infectivity. These results show that the chlamydial MOMP is a complex macromolecule antigenically. Furthermore, they indicate that the antigenic unique portions of the protein are more exposed on the organisms' surface, whereas, the antigenically common portions, demonstrated by immunoblotting, may be located within the bilayer of the outer membrane.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00230-01 LMSF

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Virulence-Associated Factors of Rickettsia rickettsii

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. L. Anacker	Research Microbiologist	LMSF NIAID
OTHER:	R. N. Philip	(Retired) Medical Director	EB NIAID
	J. C. Williams	Scientist	LMSF NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION
Rickettsial Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.4	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this study is the characterization of those rickettsial constituents which permit Rickettsia rickettsii to successfully invade and multiply within the tissues of man and laboratory animals. Six strains of R. rickettsii were classified into three virulence groups on the basis of the ability of the rickettsiae to produce disease in guinea pigs. Differences in representatives of these virulence classes were demonstrated by radioimmune precipitation of extracts of the rickettsiae surface-labeled with ^{125}I.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00231-01 LMSF

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biology of Relapsing Fever Borrelia spp.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. G. Barbour	Senior Staff Fellow	LMSF	NIAID
OTHER:	R. C. Judd	Staff Fellow	LMSF	NIAID
	L. M. Mayer	Expert (Microbiology)	LMSF	NIAID
	H. G. Stoenner	(Retired)		

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.4	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Borreliae undergo antigenic variation during the course of relapsing fever infection. Several serotypes are found among the progeny of a single borrelia inoculated into an experimental animal. We are studying the mechanism of antigenic shifts in these spirochetes. Our analysis of Borrelia hermsii lysates with sodium dodecyl sulfate-polyacrylamide gel electrophoresis has revealed a major protein that varies in apparent molecular weight between serotypes. Using both polyclonal antisera and monoclonal antibodies, we have shown that these variable proteins are also serotype-specific antigens. We are purifying the variable proteins for further biochemical characterization and employing recombinant DNA techniques to identify the gene or genes involved in antigenic variation.

PHS-6040

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00232-01 LMSF

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biology of Ixodes spp. Tick Spirochetes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. G. Barbour	Senior Staff Fellow	LMSF NIAID
OTHER:	W. Burgdorfer	Research Entomologist (Med)	EB NIAID
	S. F. Hayes	Bio Lab Technician	OB NIAID

COOPERATING UNITS (if any)

Dr. Allen C. Steere, Department of Medicine, Yale University School of Medicine, New Haven, CT

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 1.2	PROFESSIONAL: 0.7	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Investigators at Rocky Mountain Laboratories have recently discovered a spirochete in Ixodes dammini, I. pacificus, and I. ricinus ticks (see Project No. Z01 AI 00268-01 EB by Dr. Willy Burgdorfer). We have demonstrated high concentrations of antibodies to a strain of I. dammini-associated spirochete in the blood of patients with Lyme disease or erythema chronicum migrans. We are studying the biology of these spirochetes and the relationship between them and Lyme disease. We have to date: (1) formulated a cell-free medium for in vitro cultivation of these spirochetes; (2) isolated and propagated spirochetes from both I. dammini and I. ricinus; (3) raised both polyclonal antisera and monoclonal antibodies against an I. dammini-associated spirochete; and (4) identified major spirochetal antigens to which Lyme disease patients form antibody.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00233-01 LMSF

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Structural Analysis of the Major Outer Membrane Protein of Chlamydia trachomatis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. D. Caldwell	Expert (Microbiology)	LMSF NIAID
	R. C. Judd	Staff Fellow	LMSF NIAID

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.4	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Comparative structural analysis of the MOMP isolated from different Chlamydia trachomatis strains is being done using two different peptide mapping techniques. MOMPs isolated from either ^{14}C-intrinsically radiolabeled chlamydiae or lactoperoxidase mediated surface-radioiodinated organisms were digested with Staphylococcus aureus V8 protease and the peptide fragments produced displayed by SDS-PAGE or they were digested with alpha-chymotrypsin and the peptides analyzed following separation by high-voltage electrophoresis and thin-layer chromatography. The results show that considerable structural homology exists among different C. trachomatis MOMPs, however, distinct differences in primary structure were found. These differences were most obvious in peptide maps of MOMPs isolated from surface-iodinated organisms suggesting that the exposed portions of the MOMP may differ antigenically and are possibly the determinants that elicit the formation of C. trachomatis serotyping antibodies. Currently, the surface peptide fragments identified by mapping analysis are being isolated by high-pressure liquid chromatography. Antisera will be raised against these isolated peptides in order to define their immunological properties.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00234-01 LMSF

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Macrophage Interaction with Intracellular Parasites

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Hackstadt	Staff Fellow	LMSF NIAID
OTHER:	H. Caldwell	Expert (Microbiology)	LMSF NIAID
	A. Liss	Expert (Molecular Biology and Bacteriology)	LMSF NIAID

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Microbial Structure and Function

SECTION
Rickettsial Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is description of mechanisms of bacterial evasion and/or subversion of the killing mechanisms of phagocytic cells by intracellular and cell-associated parasites. Both Chlamydia psittaci and Coxiella burnetii replicate within guinea pig peritoneal macrophages (PEC), thus are able to avoid the antimicrobial mechanisms of these phagocytic cells. Neither induces macrophage oxidative killing mechanisms when assessed by stimulation of chemiluminescence or hexose monophosphate shunt activity. Mycoplasma pulmonis, a surface-associated parasite, induced in rat PEC a chemiluminescent burst equal in peak height to that induced by either Pseudomonas aeruginosa or Staphylococcus aureus. In contrast, mouse or guinea pig PEC not only failed to respond with an oxidative burst to M. pulmonis, but were inhibited in their response to subsequent challenge with S. aureus. This inhibition was both multiplicty and energy dependent.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00235-01 LMSF

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Structure and Function of Gonococcal Lipopolysaccharide

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Hitchcock	Staff Fellow	LMSF NIAID

COOPERATING UNITS (if any)
Dr. David Morrison, Emory University, Atlanta, Georgia

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	0.7	0.7

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is comprised of studies relating to the structure and function of gonococcal lipopolysaccharide (LPS), with particular emphasis on the role of LPS in the pathogenesis of gonorrhea. Studies have focused on the molecular heterogeneity of LPS as analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the interaction of proteins and lipopolysaccharide, the biochemical analysis of LPS from several prototype strains of Neisseria gonorrhoeae, the production and characterization of avian anti-LPS antisera and analysis of the role of LPS in gonococcal infections of primary chick embryo tissue culture.

PHS-6040

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00236-01 LMSF

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Studies on the Biology of Contagious Equine Metritis Organism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Hitchcock	Staff Fellow	LMSF NIAID

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.3	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project concerns characterization of the surface components of a recently defined veterinary pathogen: the causative agent of contagious equine metritis (CEM). CEM is a sexually transmitted venereal disease of horses; the etiological agent is an unclassified gram-negative diplococcus which shares several morphological and biochemical characteristics with *Neisseria gonorrhoeae*. In addition, the disease profile of CEM bears some resemblance to gonorrhea in humans. Studies have focused on the (1) formulation of a clear-typing medium which provides for adequate growth and subsequent (2) evaluation of colony morphology on this medium, (3) evaluation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the constituents of the whole cell lysates as well as surface iodinated components, and (4) preliminary evaluation of the molecular nature of lipopolysaccharide as determined by SDS-PAGE of proteinase-K digested whole cell lysates.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00237-01 LMSF

PERIOD COVERED
October 1, 1981, to August 31, 1982

TITLE OF PROJECT (80 characters or less)
Structural Analysis of Gonococcal Outer Membrane Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. C. Judd	Staff Fellow	LMSF NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.9	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The technique of ^{125}I-labeled peptide mapping has been improved and used to study both the primary structural relationships and surface exposure of the outer membrane proteins I, II, and III of several strains of *Neisseria gonorrhoeae*. In addition, the technique of high-performance liquid chromatography has been used both to analyze structural relationships and to preparatively isolate ^{125}I-peptides of interest for further structural, topographical, and immunological characterization.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00238-01 LMSF

PERIOD COVERED

October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Bordetella pertussis and *Bordetella bronchiseptica*: Outer Membrane Structure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Peppler	Expert (Microbiology)	LMSF NIAID

COOPERATING UNITS (if any)

Dr. David Bemis, University of Tennessee, Knoxville; and Dr. Gail Cassell, University of Alabama, Birmingham.

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NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.3	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was established to apply the knowledge and techniques acquired while assessing virulence-associated components in *B. pertussis* to *B. bronchiseptica*. Isogenic sets of colonial phenotype variants are selected, cloned, and compared on three media for phenotype stability. Particular attention is paid to flagella expression, assessed by light, and scanning electron microscopy, and to ^{125}I-surface labeling and lipopolysaccharide profiles on SDS-PAGE. Antisera are raised in mice to determine "phenotype specific" components by double diffusion, radio immune precipitate, and transfer-blotting techniques. Growth of different phenotypes in cell culture correlates pathogenicity with cytotoxicity. Both the in vitro cell culture system and in vivo infection of rats are used to assess the relative virulence of different strains and their phenotypes. By conducting studies with isogenic sets of phenotypes from different strains, the mechanisms and attributes of *B. bronchiseptica* infections can be studied in a more genetically controlled manner.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00239-01 LMSF

PERIOD COVERED
October 1, 1981, to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Gonococcal Surface Proteins' Immunochemical Characteristics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Swanson	Chief	LMSF NIAID
OTHER:	O. B. Barrera	Microbiologist	LMSF NIAID

COOPERATING UNITS (if any)
Dr. M. Blake, Rockefeller University, New York, and Dr. M. Tam, Genetic Systems, Seattle, Washington

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Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An immunoprecipitation method to detect gonococcal surface protein antigens was devised. This method along with immunoblotting was used to study the comparative antigenicities of protein III and protein II constituents of gonococci. For all strains examined, protein III moieties appeared antigenically identical. This confirms other studies in this lab in which identical ^{125}I-peptides were found for all protein III constituents from diverse strains (Judd, report Z01 AI 00237-01 LMSF). On the other hand, protein(s) II generally were antigenically distinct when assayed either by immunoprecipitation (with polyspecific or monospecific sera) or immunoblotting (with polyspecific sera). However, high degrees of antigenic relatedness were found for all protein(s) II when assayed by immunoblotting with monospecific sera raised against detergent-solubilized, isolated protein II material. These studies suggest that gonococci are capable of presenting a bevy of antigenic facades to their human host during gonococcal infections.

Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
1982 Annual Report
Table of Contents

Z01 AI Project Number		Page
Summary		28-1
00074-10	Genetically Controlled Mechanisms of Recovery from Friend Virus-Induced Leukemia - Chesebro	28-4
00260-01	Role of Endogenous and Recombinant Murine Retroviruses in Leukemia and Differentiation - Chesebro	28-5
00072-11	Role of Host and Viral Factors in Resistance to Rabies Virus Infection - Lodmell	28-6
00261-01	Immunological Aspects of Neurovirology - Lodmell	28-7
00073-17	Mechanisms of Immunity and Immunopathology Related to Cellular and Humoral Immunity - Coe	28-8
00262-01	Role of Pentraxins in Acute and Chronic Pathology - Coe	28-9
00085-05	Pathogenesis of Aleutian Disease Virus Infection - Bloom	28-10
00263-01	Structure and Function of the ADV Genome - Bloom	28-11
00086-05	Mechanisms of Resistance to Graft Versus Host Disease - Portis	28-12
00264-01	Structure and Function of Endogenous Murine C Type Viral Products Expressed by Differentiating Hematopoietic Cells - Portis	28-13
00199-03	Immunobiology of Aleutian Disease - Race	28-14
00265-01	Immunobiology of Scrapie Virus Infection - Race	28-15
00266-01	Genetic Structure of Murine Retroviruses - Evans	28-16
00267-01	Endogenous Murine Retroviruses and Leukemogenesis - Cloyd	28-17

Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1981 to September 30, 1982

RESEARCH HIGHLIGHTS

1. Recombinant MCF viruses from mice inoculated with Friend or Moloney murine leukemia viruses appeared to be derived from different endogenous loci. Identification of these loci has been carried out by oligonucleotide fingerprinting of recombinant viruses and by serological typing of their protein products with monoclonal antibodies.

2. Target cells for two different MCF murine leukemia viruses have been characterized. Two thymotropic leukemogenic viruses appeared to infect thymic lymphocytes rather than stromal cells, and splenotropic viruses infected splenic B lymphocytes.

3. Effect of virus spread in maintenance and recovery from Friend leukemia has been established by use of monoclonal antibodies. Passive administration of monoclonal IgG_{2a} anti-Friend virus gp70 antibodies prevented virus spread and allowed overgrowth of leukemia cells which did not release virus.

4. Multiple dominant non-H-2 genes from DBA/2 mice cause resistance to clinical graft-vs-host disease. The mechanism of these resistance effects may be related to recognition of endogenous murine leukemia virus proteins expressed on hemopoietic cells.

5. Monoclonal antibodies to Aleutian disease virus proteins can distinguish among virus strains. Utah I, Gorham and Pullman strains of ADV have differing biological activities *in vivo*, and can now be separately identified with monoclonal antibodies.

6. Three segments comprising most of the Aleutian disease virus genome have been molecularly cloned.

7. Spontaneous recovery from spinal cord infection by rabies virus has been seen in several mouse strains.

8. Hamster female protein has been identified in amyloid deposits in hamster liver.

ADMINISTRATIVE REPORT

Dr. Bent Aastad from the Royal Veterinary and Agricultural University of Copenhagen, Denmark has joined the laboratory as a Visiting Associate. He will work on Aleutian disease virus.

Dr. Richard Morrison from the University of Oklahoma will join the LPVD as a Staff Fellow working on Friend leukemia virus.

Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1981 to September 30, 1982

HONORS AND AWARDS

Editorial Boards of Journals:

Dr. B. Chesebro

Associate Editor - Journal of Immunology

Dr. D. L. Lodmell

Associate Editor - Journal of Immunology

Professional Posts:

Dr. B. Chesebro - Adjunct Professor - Department of Microbiology
Montana State University, Bozeman

Dr. M. E. Bloom - Faculty Affiliate, Department of Microbiology,
University of Montana, Missoula

Dr. J. E. Coe - Faculty Affiliate, Department of Microbiology,
University of Montana, Missoula

Dr. D. L. Lodmell - Faculty Affiliate, Department of Microbiology,
University of Montana, Missoula

Invited Lectures and Participation in Meetings and Symposia:

Dr. J. Coe - Invited Speaker, New York Academy Symposium, New York

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00074-10 LPVD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Genetically Controlled Mechanisms of Recovery from Friend Virus-Induced Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Chesebro	Chief	LPVD NIAID
OTHER:	W. J. Britt	Medical Officer	LPVD NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION
NIAID, NIH, Bethesda, MD 20205

INSTITUTE AND LOCATION

TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Three genes (Rfv-1, Rfv-2 and Rfv-3) have been identified which control host defense mechanisms required for spontaneous recovery from Friend virus-induced leukemia. Two of these genes (Rfv-1 and Rfv-2) are located in the major histocompatibility complex (H-2), and they affect the T lymphocyte response to virus-specific antigens. Rfv-2 appears to function as a dominant immune response gene. Rfv-1 acts by influencing the kinetics of development of the virus-specific T lymphocytes. Rfv-3 influences virus-specific humoral antibodies which are necessary but not sufficient for recovery. The actions of these antibodies in vivo have been studied using monoclonal antibodies derived from cells of mice recovering from leukemia.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00260-01

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Role of Endogenous and Recombinant Murine Retroviruses in Leukemia and Differentiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Chesebro	Chief	LPVD NIAID
OTHER:	W. J. Britt	Medical Officer	LPVD NIAID
	L. H. Evans	Senior Staff Fellow	LPVD NIAID
	M. W. Cloyd	Senior Staff Fellow	LPVD NIAID
	J. L. Portis	Medical Officer	LPVD NIAID

COOPERATING UNITS (if any)
None

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Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

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NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 1.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this project is the understanding of the structure and function of endogenous and recombinant murine retroviruses expressed by leukemia cells induced by inoculation of mice with Friend murine leukemia virus. To identify proteins of these viruses, we have derived a panel of monoclonal antibodies which appear to recognize the endogenous portions of the envelope proteins of recombinant viruses. These antibodies have been used in a rapid test to identify different groups of recombinant viruses and to distinguish these from non-recombinant viruses. Furthermore, some of these antibodies recognize cell-surface molecules of normal uninfected fetal hematopoietic cells. The possible roles of these endogenous gene sequences in differentiation and leukemogenesis are being studied.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00072-11 LPVD

PERIOD COVERED October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Role of Host and Viral Factors in Resistance to Rabies Virus Infections in Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: D. L. Lodmell	Scientist Director	LPVD NIAID

COOPERATING UNITS (if any)

None

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Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.75	.5	.25

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Genetic studies have shown that inbred mice vary in their susceptibility to intraperitoneally-inoculated street rabies viruses. SJL, CBA, DBA/2 and BALB/c mice are highly resistant, whereas A.SW/Sn and A/WySn mice are extremely susceptible. Inoculation of F_1 hybrids and backcross mice indicate resistance is dominant and that susceptibility is inherited as either one or two genes. Clinical disease is markedly different in the resistant strains of mice. Less central nervous system involvement with minor paralysis is present in SJL and CBA mice, whereas DBA/2 and BALB/c mice develop severe illness with paralysis. All strains of mice inoculated intracerebrally with 10 LD_{50} of virus die indicating resistance mechanism(s) and the genetic influence are functional prior to viral invasion of the principal target organ. In contrast to the different susceptibilities noted in mouse strains inoculated with street rabies viruses, all strains of mice inoculated intraperitoneally with highly virulent fixed rabies virus die. Characterization of host genetically regulated defense mechanisms(s) and virus virulence factor(s) are in progress.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00261-01 LPVD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immunological Aspects of Neurovirology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: D. L. Lodmell	Scientist Director	LPVD NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

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INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.75	.5	.25

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major objective of these studies is to delineate the importance of immune responses within the central nervous system of mouse strains which vary in their resistance to neurotropic rabies virus. The development, characterization and role of cytotoxic and neutralizing antibodies and cytotoxic T cells will be monitored in the cerebrospinal fluid. In conjunction with these immunologic studies, the role of host-cell viral receptors, cell tropisms and maturation of virus within dissociated spinal cord cultures containing differentiated neurons will be assessed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00073-17 LPVD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mechanisms of Immunity and Immunopathology Related to Cellular and Humoral Immunity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. E. Coe	Medical Officer	LPVD NIAID
OTHER:	W. J. Hadlow	Research Veterinarian	EB NIAID

COOPERATING UNITS (if any) Dr. Henry Gewurtz, Rush Medical College, Chicago, IL; Dr. David Briles, Dept. Cell. Immunobiol., Univ. Alabama, Birmingham, AL; Dr. Kamal Ishak, AFIP, Washington, D. C.; Dr. John Sogn, NIAID, Bethesda, MD; Dr. Henry Slayter, Sidney Farber Cancer Inst., Boston, MA; Dr. S. Margossian, Bronx, NY

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Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

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NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	.5	.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have been studying inflammation and immunity in the Syrian hamster and from the aspect of humoral and cellular immunity. The hamster spleen is deficient in both humoral and cellular responses when compared to peripheral lymph node. A cell population (nylon wool adherent) normally present in spleen was capable of suppressing antigen specific proliferation. IgG_1 and IgG_2 antibody synthesis also appears suppressed in the intact spleen. However, the presence of sensitized precursor cells in spleen were detectable by in vitro culture or transfer to x-irradiated recipients.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00262-01 LPVD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Role of Pentraxins in Acute and Chronic Pathology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. E. Coe	Medical Officer	LPVD NIAID
OTHER:	W. J. Hadlow	Research Veterinarian	EB NIAID

COOPERATING UNITS (if any) Dr. Henry Gewurtz, Rush Medical College, Chicago, IL: Dr. David Briles, Dept. Cell. Immunobiol., Univ. Alabama, Birmingham, AL: Dr. Kamal Ishak, AFIP, Washington, D. C.; Dr. John Sogn, NIAID, Bethesda, MD; Dr. Henry Slayter, Sidney Farber Cancer Inst., Boston, MA; Dr. S. Margossian, Bronx, NY

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1.0	.5	.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A homolog of human C-reactive protein (CRP) and amyloid P-component (AP) is present in the Syrian hamster as a sex limited protein called female protein (FP). FP functionally is similar to both CRP and AP as it has a Ca^{++} dependent phosphorylcholine binding site and is a component of hamster amyloid. FP also is involved in the inflammatory response, however, changes in serum concentration are sex limited. Catabolism of FP is similar in both sexes, although differences in distribution suggest specific transport or receptor mechanisms.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00085-05 LPVD

PERIOD COVERED October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Pathogenesis of Aleutian Disease Virus Infection

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. E. Bloom	Medical Officer	LPVD NIAID
OTHER:	R. E. Race	Veterinary Officer	LPVD NIAID

COOPERATING UNITS (if any)

None

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Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	.5	.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is the study of Aleutian disease of mink, a persistent infection by the nondefective parvovirus, Aleutian disease virus (ADV). Peptide mapping of the ADV proteins demonstrate that p85 and p75 (the two capsid proteins) have extensive peptide homology but that the nonvirion p71 is not related to the two virion proteins. This is the first demonstration in parvoviruses of a virus-induced non-virion protein. Viral expression in permissive (feline) and nonpermissive (mink) cells has been compared and the results indicated that all three proteins (p85, p75 and p71) were expressed in both cell types, although no infectious virus production in mink cells was demonstrable. This system is under further study to determine at what level virus production is blocked in nonpermissive cells.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00263-01 LPVD

PERIOD COVERED October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Structure and Function of the ADV Genome

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. E. Bloom	Medical Officer	LPVD NIAID
OTHER:	C. F. Garon	Head, Research Microbiologist	EM NIAID
	L. W. Mayer	Expert (Microbiologist)	LMSF NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 1.1	PROFESSIONAL: .6	OTHER: .5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to define the genome structure of the non-defective parvovirus, Aleutian disease virus (ADV). A double stranded intracellular form of ADV DNA analogous to replicative form DNA of other parvovirus has been isolated. This DNA has been mapped with restriction endonucleases. The restriction map differs completely from those reported for other parvoviruses. Three segments of the DNA have been molecularly cloned (0.05-0.15 map units, 0.15-0.56 map units and 0.56-0.86 map units) in bacterial systems. Plans are to clone the remaining DNA segments, and use these reagents to compare DNA from various isolates of ADV that differ in their virulence for mink.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00086-05 LPVD

PERIOD COVERED October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Mechanisms of Resistance to Graft Versus Host Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. L. Portis	Medical Officer	LPVD NIAID
OTHER:	M. W. Cloyd	Senior Staff Fellow	LPVD NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	.5	.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Acute graft versus host disease (GVH) can be induced in unirradiated adult (B6 X DBA/2)F_1 by the inoculation IV of 60-100 X 10^6 B6 spleen cells. GVH is not seen, however, in F_1 recipients of as many as 200 X 10^6 DBA/2 spleen cells. This resistance to acute GVH is abrogated by irradiation of the recipient prior to donor cell inoculation suggesting that the recipient participates in this apparent resistance to GVH. Genetic studies have revealed that this resistance phenomenon is not H-2 haplotype specific but instead is influenced by multiple dominant non-H-2 genes of the donor. Inoculation of small numbers (20-30 X 10^6) B6 or large numbers (60-100 X 10^6) DBA/2 donor cells into F_1 recipients was found to induce the production by the recipients of antibodies to a wide variety of MuLV. These antibodies react with viral envelope-like determinants expressed by lymphoid and hematopoietic cells of the F_1 and both parental strains. The possible role of these antibodies in the genesis of GVH is being studied.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00264-01 LPVD

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Structure and Function of Endogenous Murine C Type Viral Products Expressed by Differentiating Hematopoietic Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. L. Portis	Medical Officer	LPVD NIAID
OTHER:	B. Chesebro	Chief	LPVD NIAID
	W. J. Britt	Medical Officer	LPVD NIAID
	L. H. Evans	Senior Staff Fellow	LPVD NIAID

COOPERATING UNITS (if any) Dr. C. Melief, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, The Netherlands; Dr. C. Moroni, Friedrich Meischer-Institut, Basel, Switzerland; Dr. J.-P. Levy, Hopital Cochin, Paris, France

LAB/BRANCH
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	.5	.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Hybridoma cell lines have been recovered from spleens of adult F_1 mice inoculated with parental spleen cells. These lines produce antibodies which react with viral envelope determinants of a wide variety of endogenous MuLV. Monoclonal antibodies from (B6 X D2)F_1 mice inoculated with D2 spleen cells react predominantly with endogenous xenotropic MuLV. Antibodies recovered from (B6 X D2)F_1 recipients of B6 cells and (NFS X AKR)F_1 recipients of AKR cells react with determinants expressed by MCF viruses but not with any of the xenotropic, endogenous ecotropic or exogenous ecotropic MuLV in our panel. Several of these antibodies react with a gp70 molecule expressed on the membrane of a small population of cells (1-2%) in the spleen and bone marrow of adult mice and up to 70% of the cells from fetal liver. These antibodies appear to define a group of determinants unique to the erythroid lineage. The viral encapsidation of the relevant sequences appears to be under rather stringent control, as to date no infectious virus has been recovered from mouse tissues which expresses the entire spectrum of "erythroid specific determinants".

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PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immunobiology of Aleutian Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Race	Veterinary Officer	LPVD NIAID
OTHER:	B. Chesebro	Chief	LPVD NIAID
	M. E. Bloom	Medical Officer	LPVD NIAID

COOPERATING UNITS (if any)

None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this project is to define immune mechanisms and viral characteristics important in the pathogenesis of Aleutian disease (AD). Monoclonal antibodies were used to study antigenic differences among ADV isolates. Antibodies with three predominant patterns of reactivity have been identified. 1) those that react with viral antigens extracted from tissues of infected mink, 2) those that react with viral antigens purified from ADV infected cell cultures, and 3) those that react with virus from either source. Antibodies recognizing viral antigens purified from ADV infected cell cultures recognize the major viral structural proteins of ADV while the viral associated antigen recognized by monoclonal antibodies reacting with tissue extract has not been determined. These reagents allow more precise characterization of ADV isolates than has been possible previously. We are also using them as probes to localize viral antigen in tissues of infected mink. Spleen, liver, lymph node and kidney from infected mink have been examined so far but as yet no viral antigen has been demonstrated. Techniques will be modified and further attempts made in this regard. Additional monoclonal antibodies will also be used and new ones developed.

PHS-6040
(Rev. 2-81)

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PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immunobiology of Scrapie Virus Infection

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Race	Veterinary Officer	LPVD NIAID
OTHER:	B. Chesebro	Chief	LPVD NIAID

COOPERATING UNITS (if any)

None

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Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

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TOTAL MANYEARS: .5	PROFESSIONAL: .5	OTHER: 0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The immediate goal of this project is to establish high titered scrapie infected tissue culture cell lines. Scrapie is a naturally occurring spongiform encephalopathy of sheep and goats which causes clinical and pathological changes similar to those of Creutzfeldt-Jakob and Kuru diseases of man. Scrapie grows to high titer in mouse lymphoid tissue and brain but has never been passaged at high titer in any in vitro system. Adaptation of the agent to cell culture will permit detailed characterization of the agent(s) and provide a system for better definition of the pathogenesis of these diseases. We have utilized two approaches designed to provide infected in vitro cell lines. In one approach, scrapie infected mice are injected with one of several splenotropic tumor cell lines. Once tumors are established in vivo, they are explanted, maintained in vitro and assayed for the presence of scrapie agent. The second approach utilizes hybridoma technology. Spleen cells from scrapie infected mice are fused with a myeloma cell line. If a scrapie infected cell was a partner in the fusion, persistently infected lines might result. Due to the long incubation period of scrapie in mice (>4 months), results of these recent experiments are not available.

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PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Genetic Structure of Murine Retroviruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. H. Evans	Senior Staff Fellow	LPVD NIAID
OTHER:	B. Chesebro	Chief	LPVD NIAID
	M. W. Cloyd	Senior Staff Fellow	LPVD NIAID

COOPERATING UNITS (if any)
None

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Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

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NIAID, NIH, Bethesda, MD 20205

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The general aim of this project is the elucidation of genetic structural features of murine retroviruses which are relevant to mechanisms of viral replication, viral recombination, and viral oncogenicity. Current studies include the identification of cellular genetic sequences which undergo recombination with murine leukemia viruses in infected mice. The studies are directed at identifying cellular sequences which recombine with viruses of different oncogenic activity (e.g., lymphatic leukemia vs. erythroleukemia) as well as identifying virally acquired cellular sequences from different mouse strains. The initial findings demonstrate that an erythroleukemia virus and a lymphatic leukemia virus selectively recombine with different sets of endogenous proviral sequences in NFS mice. These studies are currently being extended to other mouse strains and leukemia viruses. In addition to the above studies, an assay for murine retroviruses has been developed which enables one to quantitate and recover specific viruses in complex virus mixtures. The assay, which utilizes monoclonal antibodies to detect virus-encoded cell surface antigens, is designed for the isolation of endogenous viruses from mice, the isolation of viral mutants, and in vitro recombination studies.

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PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Endogenous Murine Retroviruses and Leukemogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. W. Cloyd	Senior Staff Fellow	LPVD NIAID
OTHER:	B. Chesebro	Chief	LPVD NIAID
	L. H. Evans	Senior Staff Fellow	LPVD NIAID

COOPERATING UNITS (if any) Dr. Sisir Chattopadhyay, NCI, NIAID, Bethesda, MD; Dr. Janet Hartley, LVD, NIAID, Bethesda, MD; Dr. W. Rowe, LVD, NIAID, Bethesda, MD; Dr. C. Kozak, LVD, NIAID, Bethesda, MD; Dr. P. Hoffman, Johns Hopkins Univ., Baltimore, MD

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Two interrelated subjects are under investigation: 1) characterization of the types, properties and functions of endogenous retroviruses in mice and the mechanisms of their regulation, expression and, in the case of the recombinant (MCF) viruses, their generation, and 2) determination of the elements and processes involved in development of murine lymphoma. These projects, utilizing biological, genetic, serological, and molecular approaches, have helped to unravel some of the details of leukemogenesis in AKR mice and have partially defined the biology of MCF viruses. Recent work has revealed that MCF viruses can have restricted tropisms for lymphocyte subpopulations in vivo and that there are close associations between cellular differentiation and MCF biology. A dominant antigen of certain MCF viruses was found to be conformationally determined using monoclonal antibodies. This was the first report of a major antigen of murine retroviruses being conformational in nature. With others, the existence of MCF-like env gene sequences in chromosomal DNA of mice has been demonstrated. Some of these sequences have been further studied in newly generated panels of MCF viruses. It was found that distinct endogenous sequences recombine specifically with different ecotropic viruses in generating MCF's.

EPIDEMIOLOGY BRANCH
Rocky Mountain Laboratories
Hamilton, Montana
1982 Annual Report
Table of Contents

Z01 AI Project Number		
Summary		29- 1
00061-20	Natural History of Tick-Borne Rickettsial Agents and Their Public Health Significance - Burgdorfer	29- 8
00063-12	Immune Responses to Rickettsial Infections - Peacock and Thomas	29- 9
00065-09	Antigens and Classification of the Rickettsiae - Philip and Anacker	29-10
00069-21	Systematics and Vector Relationships of Certain Parasitic Arthropods - Clifford	29-11
00080-14	Tickborne Disease Agents - Yunker (terminated)	29-12
00081-21	The Epidemiology of Human Infections of Special Interest - Philip	29-13
00082-21	Relation of Viruses to the Genesis of Chronic Disease - Hadlow	29-14
00084-13	Vector-Pathogen Relationships - Yunker	29-15
00200-03	Immunobiology of Relapsing Fever - Stoenner	29-16
00268-01	Ecology of Lyme Disease and Related Disorders	29-17

Annual Report
Epidemiology Branch
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1981 to September 30, 1982

RESEARCH HIGHLIGHTS

The following sections summarize this year's developments in our research program and discuss their significance in relation to the epidemiology and ecology of diseases under investigation. Some of the findings were reported in 27 publications and an additional 12 papers have been accepted by scientific journals.

RICKETTSIOSES

Although many of our collaborative studies with outside agencies have been terminated, field, microbiological and clinical materials continue to be received from areas where rickettsial diseases, especially Rocky Mountain spotted fever and Q fever, are measurable public health problems. As in previous years, we functioned as a World Health Organization Center for Rickettsial Reference and Research and provided reference reagents, guidance and training to investigators and staff of various domestic and foreign health agencies. Although our research efforts concentrated toward biochemical characterization and identification of nonpathogenic and pathogenic rickettsiae, we continued studies pertaining to clarification of the fundamental ecologic interrelationships between rickettsiae, their tick vectors and hosts. Research highlights include the following observations:

The results of the recently terminated 4-year Ohio study indicate that the newly described (see Annual Report 1980/81) *Rickettsia belli* is widely distributed in that state, that *R. montana* is localized to counties bordering the Ohio River, and that *R. rickettsii* exists in certain sharply localized pockets in central and northern Ohio. Absence in dogs of antibodies to *R. belli* in areas where this rickettsia is abundant suggests that dogs are refractory to this agent. (Philip)

Preliminary SDS-PAGE and electrofocusing studies of the California *R. rickettsii*-like isolates from *Dermacentor occidentalis* show no differences in protein composition and suggest that the recently proposed subspecies, *R. rickettsii californica*, is not justified. (Philip)

Quantitative studies on interference between spotted fever group rickettsiae suggest that the efficacy of this phenomenon depends on the degree of initial rickettsial infection. Presently monoclonal antibodies are being produced in the hope that they may be capable of distinguishing between the closely related rickettsial species and thus may be applicable in determining the mechanism(s) of interference. (Burgdorfer, Todd)

Electron microscopy of R. rickettsii revealed that reversible structural modifications of the microcapsular and slime layers are linked to physiologic changes in the tick host and correlate with "reactivation", i.e., restoration of pathogenicity and virulence of the spotted fever agent. In engorged ticks, the microcapsular layer consists of a discrete layer approximately 16 nm thick and is composed of globular subunits with a periodicity of approximately 10 nm. The slime layer external to the microcapsular layer forms a discrete electron lucent zone around the rickettsia. In starved ticks, the microcapsular and slime layers are shed to form stringy, shredded, and somewhat flocculent strands. Incubation at 37°C or feeding results in the restoration of the discrete layers. (Hayes, Burgdorfer)

High-voltage electron microscopy (HVEM) revealed the presence of two types of fibers within the halozone of R. rickettsii. One type appears as extensions of the rickettsial outer membrane, the other as microfilaments from the host cell attached to the rickettsiae. In addition, individual rickettsiae within the same infected cell were shown to be joined to each other by connections formed from the rickettsia's outer membrane. These hitherto unrecorded structures may serve important functions in the host/parasite relationships. (Todd, Burgdorfer)

Chronic Q fever endocarditis, well-documented in Great Britain and Australia, has rarely been diagnosed in the U.S. but is gaining increased medical attention as a result of Q fever resurgence in this country. In studying the antibody responses to Coxiella burnetii phase I and phase II in patients with the primary or the chronic disease, it was found that the indirect microimmunofluorescence test with specific anti-human IgM, IgG, and IgA conjugates discriminated between primary Q fever and chronic Q fever of either heart valves or liver. The IFA, microagglutination or CF tests were useful in discerning primary infection from chronic infection. High phase II, IFA anti-IgG and IgM antibody titers were characteristics of the granulomatous hepatic disease. High phase I and phase II IFA anti-IgG titers characterized endocarditis. The presence of specific anti-C. burnetii phase I and phase II antibody titers of the IgA class was diagnostic for Q fever endocarditis. Such antibodies were not found in sera of primary or hepatic Q fever patients. During these studies, 5 isolations of C. burnetii phase I were made from aortic valve vegetations and 2 isolations from liver punch or bone marrow biopsy material. (Peacock)

SYSTEMATICS OF TICKS

In spite of plans to have RML's tick collection and associated personnel transferred to the Smithsonian Institution in the summer of 1983, systematic treatment including taxonomy, biology, ecology, and colonization of parasitic acarina of medical importance continued. Studies on the Haller's organ of the genus Argas, as well as those on the Ornithodoros capensis, Ixodes oldi, and Rhipicephalus complexes resulted in three papers dealing with the Haller's organ of the genus Argas, one paper on the description of a new tick species closely related to O. denmarki, and one paper on the description of 5 new

Systematics of ticks (cont'd)

tick species of the *I. oldi* complex from Africa. In addition, a new species of *Ixodes*, *I. eastoni*, from South Dakota was described. (Clifford, Keirans)

In collaboration with Dr. Centurier from the Institute of Tropical Medicine, University of Munich, West Germany, a project was initiated to determine the presence of isoenzymes in ticks of the *R. sanguineus* complex. Comparative analysis of the isoenzyme components may be applicable to speciation of taxonomically closely related ticks. (Clifford, Centurier)

From May until November 1981, approximately 10,000 new tick records were entered into the NIH computer and transferred to the Smithsonian data bank.

VECTOR-PATHOGEN RELATIONSHIPS

The two recently established continuous cell lines from *Rhipicephalus sanguineus* (see Annual Report 1980/81) were found to be composed of cells resembling tick hemocytes and were shown to propagate rickettsial species of various serological and biological groups such as *R. rickettsii*, *R. akari*, *R. typhi*, and a newly discovered *Rickettsia* sp. from argasid ticks in South Dakota and Oklahoma. All rickettsiae showed exponential increases in titers within 2 weeks of inoculation. The fact that increases of *R. rickettsii* were 2.5 logs less in *Rh. sanguineus* cells than in *Dermacentor* cells may reflect vector relationships of this agent inasmuch as in North America *R. rickettsii* is transmitted by *Dermacentor* ticks but not by *Rh. sanguineus*.

Of the three arthropodborne Flaviviruses, yellow fever (mosquitoborne), Modoc, and Langat (tickborne), yellow fever grew to higher levels. The ability of tick cells to distinguish between closely related viruses based on vector or non-vector status has been noted before and lends credence to epidemiological evidence that Modoc virus is transmitted directly from vertebrate to vertebrate. (Yunker)

Biochemical analysis of tissue culture medium performed at zero time (brief exposure to cells) and at one week of cell growth will hopefully shed light on the utilization by tick cells of carbohydrates, amino acids, metals, proteins, and lipids and may permit improvement or simplification of tick tissue culture media. Results available so far indicate significant changes in concentrations of organic acids in both the zero-time sample and those in which cells have been grown for one week. Whereas the concentration of succinic acid increased in both samples, complete depletion of both citric and x-ketoglutaric acid was seen. A possible explanation for the increase of succinic acid is that this entity represents an end-product of tick cell metabolism. The decline of the other organic acids may indicate that these are being effectively utilized by tick cells. (Yunker)

In cooperation with investigators at Yale, serologic characterization of two new tickborne viruses - one from *Argas brumpti* in Tanzania, the other from *O. coriaceus* in California - have been completed. The former may be involved in a febrile illness of humans, the latter is suspected of being involved in epidemic bovine abortion of cattle. (Yunker)

RELATION OF VIRUSES TO CHRONIC DISEASE

These readily manipulated viral infections of animals continue to serve as useful models for studying the diverse ways viruses bring about disease after long periods. Some findings would seem to be applicable to the understanding of the pathogenesis of comparable diseases in man, as for example scrapie and Creutzfeldt-Jakob disease (CJD). Other findings, such as those on Aleutian disease, characterize pathogenic mechanisms that may explain the genesis of some chronic human disease.

Although new work related to this project was discontinued 4 years ago, experiments with CJD and scrapie are still in progress. To date, tissues from 5 of 10 persons dying of CJD have induced encephalopathy in goats. Clinically and neurohistologically, it resembles scrapie. The incubation periods in animals thus far affected have ranged from 37 to 60 months. The significance of the findings in goats is still not understood. Despite the similarities of scrapie and CJD, recent observations do not point to any epidemiologic relationship between them. (Hadlow)

IMMUNOBIOLOGY OF RELAPSING FEVER

In contrast to previous interpretation, seroconversion of the relapsing fever spirochete, Borrelia hermsii, occurs rather spontaneously and independently of the host's immune responses. This was shown after cultivation of single spirochetes became possible in Kelly's medium. Two serotypes, 7 and 21, were inoculated separately. After 15 days of growth, cultures inoculated with a single serotype 7 spirochete converted to types 6, 17, 18, 20 and 24. Similarly, cultures inoculated with one organism of type 21 converted to types 2, 7, 9, 12, 14, 15, 17, 20, 22, and 24. The conversion mechanism is still a matter of conjecture. (Stoenner)

LYME DISEASE AND RELATED DISORDERS

During the course of a rickettsial survey of ticks from Shelter Island, New York, a treponemalike spirochete was detected in and isolated from Ixodes dammini, the epidemiologically incriminated vector of the hitherto unknown agent of Lyme disease. Application of indirect immunofluorescence showed that the spirochete was involved in the etiology of the disease. Although spirochetes have not as yet been recovered from patients, there is increasing convincing evidence that the organism is the cause of Lyme disease and related disorders. Spirochetes indistinguishable from those in I. dammini have also been detected in I. pacificus in Oregon and in I. ricinus in Switzerland where disease entities similar to Lyme disease are common. A preliminary report in Science (June 18, 1982) was met with excitement and enthusiasm by clinicians and treponematologists. Preliminary observations suggest a rather complex relationship of the spirochete to its tick vectors. In some ticks the agent appears to be limited to the midgut, whereas in others it causes a generalized infection including the eggs. (Burgdorfer, Barbour, Péter, Hayes)

The discovery of the Lyme disease agent has provided excellent opportunites for cooperative research between RML laboratories (see also Project No. Z01 AI 00232-01 LMSF).

Annual Report
Epidemiology Branch
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1981 to September 30, 1982

ADMINISTRATIVE REPORT

Currently, the Epidemiology Branch is manned by 6 Ph.D. level scientists, all tenured, including 4 in the Arthropod-Borne Diseases Section, and one each in Epidemiology and Histopathology. In addition, there are 5 supporting staff and one secretary assigned to the Office of the Chief. A permanent Chief of EB has still not been appointed.

During the year, Dr. R. N. Philip, Acting Chief, EB, retired (3/31/82) and his administrative duties were assigned to Dr. W. Burgdorfer. Harry Meibos retired from the Arthropod-Borne Diseases Section January 8, 1982, and the temporary appointment of Val Moore, who was added to the staff to curate the work on tick taxonomy, terminated November 27, 1981.

Dr. Olivier Péter, University of Neuchâtel, Switzerland, was appointed NIH Visiting Fellow and started his tenure January 4, 1982, to receive training in rickettsiology under the supervision of Dr. Burgdorfer. Guest workers in Dr. Clifford's unit included Dr. Claus Centurier, Institute of Tropical Medicine, Munich, West Germany, April 1-August 16, 1982, and Dr. Gustavo Lopez, El Nus Experiment Station, Antioquia, Colombia, Dec. 29, 1981 through July 2, 1982. Dr. J. D. Bezuidenhout, Veterinary Research Institute, Onderstepoort, Pretoria, South Africa, was a guest worker in Dr. Burgdorfer's unit June 8 through August 26, 1982.

Annual Report
Epidemiology Branch
Rocky Mountain Laboratories
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October 1, 1981 to September 30, 1982

HONORS AND AWARDS

The following activities reflect recognition of EB by their peers in the scientific community.

Editorial Boards of Journals:

Dr. W. Burgdorfer - Acta Tropica, Current Topics in Pathogen-Vector-Host Research, Co-Editor of Rickettsiae and Rickettsial Diseases
Dr. W. J. Hadlow - Fundamental and Applied Toxicology
Dr. C. E. Yunker - Journal of Parasitology

Drs. Burgdorfer, Clifford, Hadlow, Keirans, and Yunker reviewed manuscripts for J. Med. Entomol., J. Econ. Entomol., Ann. Entomol. Soc. Amer., J. Wildlife Dis., J. Infect. Dis., Science, Vet. Pathol., and J. Parasitol.

Professional Posts:

Dr. W. Burgdorfer - named Acting Chief, EB, RML; elected President of the American Society for Rickettsiology and Rickettsial Diseases; member of Research and Thesis Committee of the Zoological Institute, University of Neuchâtel, Switzerland

Dr. W. J. Hadlow - Adjunct Professor of Veterinary Pathology, Wash. St. Univ.

Drs. C. M. Clifford and J. E. Keirans - appointed Research Associates of the National Museum of Natural History, Smithsonian Institution, Wash., D.C.

Dr. C. E. Yunker - continued as U.S. Delegate to the International Committee on Polar Viruses.

Invited Lectures and Participation in Meetings and Symposia

Dr. W. Burgdorfer - invited to participate at National Tick Symposium April 6-8, 1982, at Kenlake State Park, Aurora, KY and present lecture "Pathological Organisms Associated with Ticks"; invited to prepare chapter on "Transovarial transmission of arthropod-borne viruses and rickettsiae" for Current Topics in Pathogen-Vector-Host Research; invited to participate at International Symposium "Erreger und Übertrager tropischer Krankheiten", Basel, Switzerland, to present paper "Erythema chronicum migrans - a tick-borne spirochetosis".

Dr. C. M. Clifford - invited to participate in the 5th Int. Congress of Acarology, Edinburgh, Scotland, Sept. 5-11, 1982.

Honors and Awards (cont'd)

Dr. C. E. Yunker - invited to present a seminar at Syntex Corp., Palo Alto, CA, on Principles and Applications of Tick Tissue and Cell Cultures; invited to present a lecture on the same topic at the 5th Int. Congress of Acarology, Edinburgh, Scotland, Sept. 5-11, 1982; invited to participate in the VI Int. Conference on Invertebrate Tissue Culture, St. Augustine, FL, June 5-10, 1983, and to present a paper on the Susceptibility of Tick Cell Lines to Arbovirus Infection; invited to participate in a symposium on Sub-Arctic and Arctic Viruses and Vectors at the IV Int. Conference on Comparative Virology, Banff, Canada, Oct. 17-22, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00061-20 EB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Natural History of Tick-Borne Rickettsial Agents and Their Public Health Significance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. Burgdorfer	Res. Entomologist (Med.)	EB NIAID
OTHER:	R. N. Philip	Medical Director	EB NIAID (Retired 1982)
	M. G. Peacock	Microbiologist	EB NIAID
	O. Péter	NIH Visiting Fellow	EB NIAID
	J. C. Williams	Biochemist	LMSF NIAID
	W. J. Todd	Senior Staff Fellow	OB NIAID
	S. F. Hayes	Biol. Lab. Tech.	OB NIAID

COOPERATING UNITS (if any) Drs. J. Anderson and L. Magnarelli, Conn. Agric. Res. Exp. Station; Dr. E. L. Snoddy, LBL, TVA; Dr. A. Aeschlimann, University of Neuchâtel, Switzerland.

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Arthropod-Borne Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 3.4	PROFESSIONAL: 1.6	OTHER: 1.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project concerns studies of Rocky Mountain spotted fever and other tick-borne rickettsial diseases in the United States and in certain other countries with emphasis on the ecology, identification, and characterization of rickettsiae and on their relationship(s) with the respective tick vectors. Source material for experimental comparative studies is obtained through collaboration with outside agencies, such as state departments, public health laboratories, hospitals, physicians, etc. The project also considers the cellular and subcellular aspects of interaction between tick-borne rickettsiae and their vectors, particularly the mechanism(s) of interference and the factors responsible for changes in the agents' pathogenicity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00063-12 EB

PERIOD COVERED October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Immune Responses to Rickettsial Infections

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. G. Peacock	Microbiologist	EB NIAID
	L. A. Thomas	Res. Microbiologist	EB NIAID
OTHER:	W. Burgdorfer	Res. Entomologist (Med.)	EB NIAID
	J. C. Williams	Biochemist	LMSF NIAID
	R. N. Philip	Medical Director	EB NIAID (Retired 1982)

COOPERATING UNITS (if any) Ruth S. Faulkner, Dept. of Microbiology, Public Health Laboratories, Halifax, Nova Scotia.

LAB/BRANCH
Epidemiology Branch (RML), Hamilton, MT 59840

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.4	OTHER: 0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study immune responses in man and experimental animals to natural and experimental rickettsial infections. Currently, studies have involved isolation and characterization of Coxiella burnetii isolates from heart valves and the characterization of the immune response in man to chronic Q fever disease. The various serological parameters were compared by the microagglutination, complement fixation and the indirect microimmunofluorescent antibody tests. These serological tests were found to adequately differentiate primary, granulomatous hepatic and chronic endocarditis Q fever disease entities in man. An enzyme-linked immunosorbent assay (ELISA) test has been developed for the diagnosis of Q fever. Particulate purified phase I and phase II C. burnetii rickettsiae and solubilized fractions of phase I were found to be practical antigens in the Q fever ELISA test.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00065-09 EB LMSF

PERIOD COVERED October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Antigens and Classification of the Rickettsiae

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. N. Philip	Medical Director	EB NIAID
	R. L. Anacker	Research Microbiologist	LMSF NIAID
OTHER:	None		

COOPERATING UNITS (if any) Scott W. Gordon, Ohio Dept. of Health, Columbus.

LAB/BRANCH Epidemiology Branch & Laboratory of Microbial Structure & Function (RML)
Hamilton, MT 59840

SECTION
Epidemiology Section and Rickettsial Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.4	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long-range objectives of this project are to develop and apply practicable procedures for classification of spotted fever- and typhus-group rickettsiae and to determine the nature and biological properties of rickettsial antigens and constituents, particularly those that are associated with pathogenicity. This part of the project focuses on differentiating serologically the kinds of rickettsiae found in arthropod vectors known to transmit rickettsial disease agents to man or are involved in the maintenance of pathogenic rickettsiae in nature with particular reference to the United States. The hemolymph test) for preliminary screening of ticks, Vero-cell culture for isolation of rickettsiae from HT-positive ticks, and the microimmunofluorescence test of mouse antisera for identification of isolates have been excellent procedures for this purpose. Representative strains of the important serotypes are being examined by other techniques for biologic characterization (see also same project number in LMSF report). This part of the project was terminated March 31, 1982 with the retirement of PI (Philip).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00069-21 EB

PERIOD COVERED October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Systematics and Vector Relationships of Certain Parasitic Arthropods

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. M. Clifford	Scientist Director	EB NIAID
OTHER:	J. E. Keirans	Res. Entomologist (Med.)	EB NIAID
	C. E. Yunker	Scientist Director	EB NIAID

COOPERATING UNITS (if any) Drs. H. Hoogstraal, NAMRU-3; D. Sonenshine and P. Homsher, Old Dominion Univ.; C. Centurier, Univ. of Munich; F. S. Lukoschus, Catholic Univ., Netherlands; Miss Jane Walker, Div. of Vet. Services, Onderstepoort; Mr. Rupert Pegram, Tick Diseases Unit, Lusaka, Zambia.

LAB/BRANCH

Epidemiology Branch (RML), Hamilton, MT 59840

SECTION

Arthropod-Borne Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 3.2	PROFESSIONAL: 2.2	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The activities of this project currently comprise four main functions: 1) Identification of ticks received from various individuals and government agencies throughout the world. Only one other institution in the world is capable of performing this service. 2) Systematic study of certain groups of parasitic arthropods. The foremost tool in systematic studies, the scanning electron microscope, has greatly aided in elucidating taxonomic concepts in acarines actually or potentially involved in transmission of disease agents. 3) Retrieval and use of tick data in the Smithsonian data retrieval system. Installation of a mini-computer-word processor system at RML to communicate with NIAID and Smithsonian computers. 4) Colonization of medically important arthropod vectors, which are furnished to outside investigators.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00080-14 EB

PERIOD COVERED October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Tickborne Disease Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. E. Yunker	Scientist Director	EB NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Epidemiology Branch (RML), Hamilton, MT 59840

SECTION
Arthropod-Borne Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Project terminated

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00081-21 EB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
The Epidemiology of Human Infections of Special Interest

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. N. Philip	Medical Director	EB NIAID
OTHER:	None		

COOPERATING UNITS (if any) Dr. Catharine Wilfert, Duke University School of Med., Durham, N.C.

LAB/BRANCH
Epidemiology Branch (RML), Hamilton, MT 59840

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project emphasizes epidemiologic investigations of infectious diseases of particular interest to the research program of this laboratory. Current investigations include studies of the etiologic relationship of various spotted fever-group serotypes to human illness, study of epidemiologic attributes pertinent to occurrence of Rocky Mountain spotted fever (RMSF) in a high incidence area, and improvement in laboratory methods for diagnosis of rickettsial infections, particularly RMSF. This project was terminated March 31, 1982 with retirement of PI (Philip).

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00082-21 EB

PERIOD COVERED October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Relation of Viruses to the Genesis of Chronic Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. J. Hadlow	Res. Veterinarian (Path.)	EB NIAID
OTHER:	None		

COOPERATING UNITS (if any) Dr. S. B. Prusiner, Dept. of Neurology, University of California, San Francisco.

LAB/BRANCH
Epidemiology Branch (RML), Hamilton, MT 59840

SECTION
Histopathology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Several naturally occurring viral infections of domestic animals are studied to learn more about the unusual host-virus interactions giving rise to diseases that evolve slowly after long incubation periods. Two diseases of greatest concern are scrapie of sheep and goats and Aleutian disease of mink. Scrapie is a degenerative disease of the brain (polioencephalopathy) caused by virus replicating in central nervous tissue. Aleutian disease is mainly a chronic disease of the kidneys that is mediated by deposits of infectious immune complexes in the glomeruli. By use of fairly simple methods of clinical observation, serology, virology, animal inoculation, and anatomic pathology, information is sought on the pathogenesis and natural history of each disease. Such information not only will help define the diseases and the mechanisms that give rise to them, but also will help determine the relation of protracted viral infection to the genesis of chronic disease in man. One such human disease, Creutzfeldt-Jakob disease, is included in the project to determine its possible etiologic relation to scrapie.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00084-13 EB

PERIOD COVERED October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Vector-Pathogen Relationships

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. E. Yunker	Scientist Director	EB NIAID
OTHER:	None		

COOPERATING UNITS (if any) Dr. K. Abdel-Wahab, Al Azhar Univ., Cairo; Dr. M. Y. Kamel, National Research Center, Cairo; Dr. A. Main, Yale Univ. School of Medicine, New Haven, CT; Dr. C. Chastel, Faculte de Medecine, Brest; Dr. M. Takahashi, Nat. Inst. Health, Tokyo; Dr. C. Centurier, Univ. of Munich.

LAB/BRANCH
Epidemiology Branch (RML), Hamilton, MT 59840

SECTION
Arthropod-Borne Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 1.7	PROFESSIONAL: 1.0	OTHER: 0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project deals with the interactions of vector-borne microorganisms, arboviruses, rickettsiae and protozoans, and cells of haematophagus arthropods, mainly ticks. Model in vitro systems are developed and applied to studies of pathogen host-range, growth dynamics, persistence and interference. After prolonged growth in these systems, pathogens are examined for changes in invasiveness, virulence, or antigenic characteristics. With overseas collaborators we are studying critical factors influencing survival of ticks and of pathogens within ticks from a biochemical, histochemical, and physiological standpoint. Two cell lines from Rhipicephalus ticks, which we recently established in continuous culture, were characterized with regard to karyology, growth characteristics and ability to support multiplication of viral and rickettsial agents. A study of the utilization of organic acids by tick cells in vitro was initiated. Serological characterization of two undescribed tickborne viruses has been completed.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00200-03 EB

PERIOD COVERED October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immunobiology of Relapsing Fever

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: H. G. Stoenner Asst. Sci. Director, NIAID (RML) (Retired 1981)
OTHER: None

COOPERATING UNITS (if any)

None

LAB/BRANCH
Office of the Scientific Director, NIAID

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is principally concerned with elucidating the pathogenesis of relapsing fever and the dynamics of seroconversion of the organism.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00268-01 EB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Ecology of Lyme Disease and Related Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. Burgdorfer	Res. Entomologist (Med.)	EB NIAID
OTHER:	A. G. Barbour	Senior Staff Fellow	LMSF, NIAID
	S. F. Hayes	Biol. Lab. Tech.	OB NIAID
	O. Peter	NIH Visiting Fellow	EB NIAID

COOPERATING UNITS (if any) Dr. J. Benach, NY State Health Dept., Stony Brook; Dr. R. S. Lane, Univ. of California, Berkeley; Dr. J. P. Davis, Dept. Health & Social Sciences, Madison, WI; Dr. A. Aeschlimann, Univ. of Neuchatel, Switzerland.

LAB/BRANCH
Epidemiology Branch (RML), Hamilton, MT 59840

SECTION
Arthropod-Borne Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 1.3	PROFESSIONAL: 1.1	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to determine the natural history of the recently discovered and isolated treponemalike causative agent of Lyme disease and related disorders (ECM of Europe). The relationship(s) between the spirochete and its various Ixodes spp. tick vectors (I. dammini, I. pacificus, I. scapularis, I. ricinus) is being determined by establishing through conventional as well as transmission and scanning electron microscopy methods a) the development of the spirochete within the ticks, and b) the mode(s) of transmission to vertebrate hosts. In cooperation with outside agences, tick/spirochete surveys are being conducted to determine prevalence of infected ticks in endemic foci. Similarly, the natural source(s) for infection of ticks is being evaluated first serologically (indirect immunofluorescence) and subsequently through recovery of the spirochete from serologically implicated hosts.

ROCKY MOUNTAIN OPERATIONS BRANCH
Rocky Mountain Laboratories
Hamilton, Montana
1982 Annual Report
Table of Contents

Introduction 30-1

General Overview 30-2

Project No.
Z01 AI 00201-03 Structural Characterization of Integrated Viral Genomes - Garon 30-3

Annual Report
Operations Branch
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1981 to September 30, 1982

Introduction

The Branch provides all services necessary to the professional staff in the pursuit of their investigations. This support covers the following areas: procurement, personnel, custodial, security, media preparations, waste disposal, glassware refinishing, photography, animal rearing and care, motor pool, operation of power plants, and full maintenance in every area except electronics.

General Overview of the Responsibilities of Operations Branch

The fiscal and procurement department manages a budget of $1,025,000. Payroll is not included in this figure. It covers only the purchase of supplies and minor equipment used in the operation of the laboratories. Timekeeping and the correction of errors in the payroll are also handled in the unit.

Personnel handles all actions and advises on personnel matters. This department also is charged with the operation of the Comprehensive Employment Training Act (CETA) in association with the local Montana State Employment Office. Through the year we have averaged two people on this program, each serving a 2-month appointment. The maximum time a person may spend on the program is 2 months. Hence, we are constantly interviewing and employing people under the program. Also handled by Personnel are persons under the following programs: Stay-in-School, Work Study, Government Summer Program, Visiting Program, and students studying for advanced degrees.

Custodial services are provided in the 5 laboratory buildings daily. Security is provided in the form of a guard on duty every night of the year.

Most of the media used in the laboratories is prepared at the lab under a contract by an outside contractor. All glassware used in the lab is cleaned and sterilized for reuse in the laboratories.

The Graphic Arts Department provides full professional services necessary in the laboratories with the exception of medical artistry.

The Animal Unit raises one strain of guinea pig, 12 strains of mice, 2 strains of hamsters, and one colony of microtus. They breed and raise approximately 90,000 animals a year. An additional 10,000 animals are purchased annually from outside sources, including mink, sheep, rabbits, mice, and hamsters. After rearing, care is provided for these animals while they are under experiment.

The maintenance department, through the power plant, provides heat, steam, air and vacuum to the laboratories. Also provided are air conditioning, compressed air, and demineralized and distilled water. A motor pool consisting of 10 vehicles is maintained. Grounds care, including snow removal, is provided.

With the exception of the electronics work, all maintenance is done by the staff. This includes plumbing, electrical, sheet metal, carpentry, and refrigeration, including ultra low temperature boxes.

Labor management work is handled by the Chief of the Branch.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00201-03 RMOB

PERIOD COVERED October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Structural Characterization of Integrated Viral Genomes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Claude F. Garon	Section Head	RMOB/NIAID
OTHER:	Stanley F. Hayes	Bio. Lab. Tech.	RMOB/NIAID
	William J. Todd	Senior Staff Fellow	RMOB/NIAID
	Melvin D. Corwin	Bio. Lab. Tech.	RMOB/NIAID

COOPERATING UNITS (if any) Malcolm A. Martin, LMM/NIAID; Takis S. Papas, NCI; Charles J. Sherr, NCI; R. Silver, FDA.

LAB/BRANCH
Rocky Mountain Operations Branch (RML), Hamilton, MT 59840

SECTION
Electron Microscopy Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 1.5	PROFESSIONAL: 0.8	OTHER: 0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Retroviruses provide a unique and important system for the study of integrative recombination since exogenously acquired genomes are integrated with high efficiency at a specific site in the viral genome, but at a large number of sites in the host chromosome. Molecular clones of several newly integrated retroviral genomes were produced in either plasmid or bacteriophage cloning vehicles using approved recombinant DNA techniques and were characterized using electron microscope heteroduplex and R-looping methods. These studies have not only provided information on the structural arrangement of both integrated viral and flanking cellular sequences but also have confirmed the presence of transformation specific sequences in normal, uninfected host cells. The major objective of these studies has been the application of physical and biochemical techniques to assess the influence of flanking cellular sequences on subsequent viral function and to define in molecular terms those events which take place during integrative recombination.

PHS-6040
(Rev. 2-81)

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Bethesda, Md. 20205

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